The Locomotory and Myotomal Musculature of the Seahorse

Hippocampus abdominalis.

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Abstract

The seahorse *Hippocampus abdominalis* is a member of the family Syngnathidae, a group characterised by small carnivorous demersal fishes with an external covering of bony plates. Another feature typical of this lineage is the presence of a prehensile tail, which is usually terminated by a modest but functional caudal fin. However, the seahorse is unique in that its myotome is designed for an exclusively non-locomotory purpose. The unorthodox functional orientation of this structure is parallelled by the exceptional vertical swimming posture, and reflects the transformation of the locomotory emphasis to the dorsal fin.

In this study, the dorsal and myotomal musculature was examined in terms of histochemical and ultrastructural evaluations. Quantitative analyses were also carried out to determine the mitochondrial content of the different fibre types, the proportions of the fibre types in the myotomal and locomotory musculature, and the respective fibre diameters. The conclusions drawn from the results of these investigations were subsequently evaluated in relation to the locomotory mode of the animal.

The investigative techniques revealed four muscle fibre types; not all of which were present in entirely homogeneous populations. These were distinguished on the basis of glycogen content, lipid content, oxidative enzyme activity and stability of myofibrillar ATPase enzymes at the light microscope level; and from the ultrastructural examinations at the cellular level.

Three principal features were noted: the complete absence of the mATPase labile slow-contracting red fibres; the lack of oxidative musculature of any kind in the myotome; and the presence of a substantial population of tonic fibres in

the myotome. These fibres were present in uniquely high proportions, and displayed a dimensional variation which has also not previously been recorded. Furthermore, they differed in terms of certain details of their ultrastructure.

The oxidative pink fibres of the dorsal fin contained the greatest proportions of mitochondria, in unusually high proportions for oxidative muscle *per se*.

Fibre diameters of the different muscle types were in all cases, significantly different. In addition, an increase in the dimensions of all fibre types with fish length was recorded.

General Introduction

Fishes are the largest and most ancient group of vertebrates, dating from the late Cambrian period at more than 500 million years old. The earliest piscean remains are limited to fragments of bone and teeth, but excavation of later Ordovician strata has produced virtually complete skeletons of fish-like vertebrates which became the principal form of life during the Devonian period- the "Age of Fishes". The geological record has provided evidence that these ancestral forms were represented by a number of diverse and often specialised types, a fact that suggests that even at this point in time, each of the primitive classes had already enjoyed a respectable antiquity (Wootton, 1990).

The major distinctive osteichthyan (bony fish) lineages of the Devonian are classified as the Actinopterygii, the Dipnoi, and the Crossopterygii. Each group includes representatives still in existence today, although evolution has ensured considerable diversification from the original forms. Of the latter two groups, which collectively form the Sarcopterygii (the fleshy finned fishes), some evolved major adaptions critical to the emergence of the terrestrial vertebrates, whereas the ray-finned actinopterygians retained their archetypal fish-like characters and gave rise to the modern bony fishes, the teleosts.

The evolution of the teleost fishes occurred during the Mesozoic era, which saw the expansion of several major radiations of phylogeny including the terrestrial groups. According to McFarland (1985), the teleosts are polyphyletic in origin; the vast majority of living representatives probably derived from four advanced halecostome holostean types before the time of the upper Cretaceous. The modern descendants of these clades are divided into four taxa of varying

size and diversity, of which the basal stock is represented by the salmoniform fishes.

The teleosts now dominate both marine and freshwater systems. They are represented by over 23,000 living species (Wootton, 1990) and account for nearly half of all vertebrates (Nelson, 1984). Of the living species 58% are marine, 41% live in fresh water, and 1% migrate between fresh and salt waters (Moyle and Czech, 1982; Bone, 1989).

The teleosts have mastered life in water, colonizing virtually every aquatic habitat covering over 70% of the earth's surface. As well as existing in the conventional domains of lakes, ponds, rivers, rock pools and the open ocean, they have overcome the challenges presented to them by desert environs, the deep sea, high altitudes, the Antarctic, and in warm waters of high alkalinity and low oxygen. Associated with this wide range of habitats and adaptations, fishes show an extraordinary variety of body shapes and life-history patterns (Nelson, 1984; Breder and Rosen, 1966). Ancient fish ancestors were typically spindle-shaped and streamlined, but evolution has seen considerable diversification of ancestral themes of form and function, although many of the modern fishes retain the traditional fusiform streamlined body shape (Bone, 1982; Lindsey, 1978) (Figure 1).

Modern fish show a profusion of locomotory types which complement the form of the individual. The variety and extent of teleost diversification evident is a consequence of countless interactions between environmental challenges and the biological characteristics of individuals in each particular lineage. Constraints imposed by the morphology and physiology of the individual relative to the specific environment effectively define the mode of life.

The physical properties of water are an influential determinant of piscine form and function because of the relationship between body form and effective

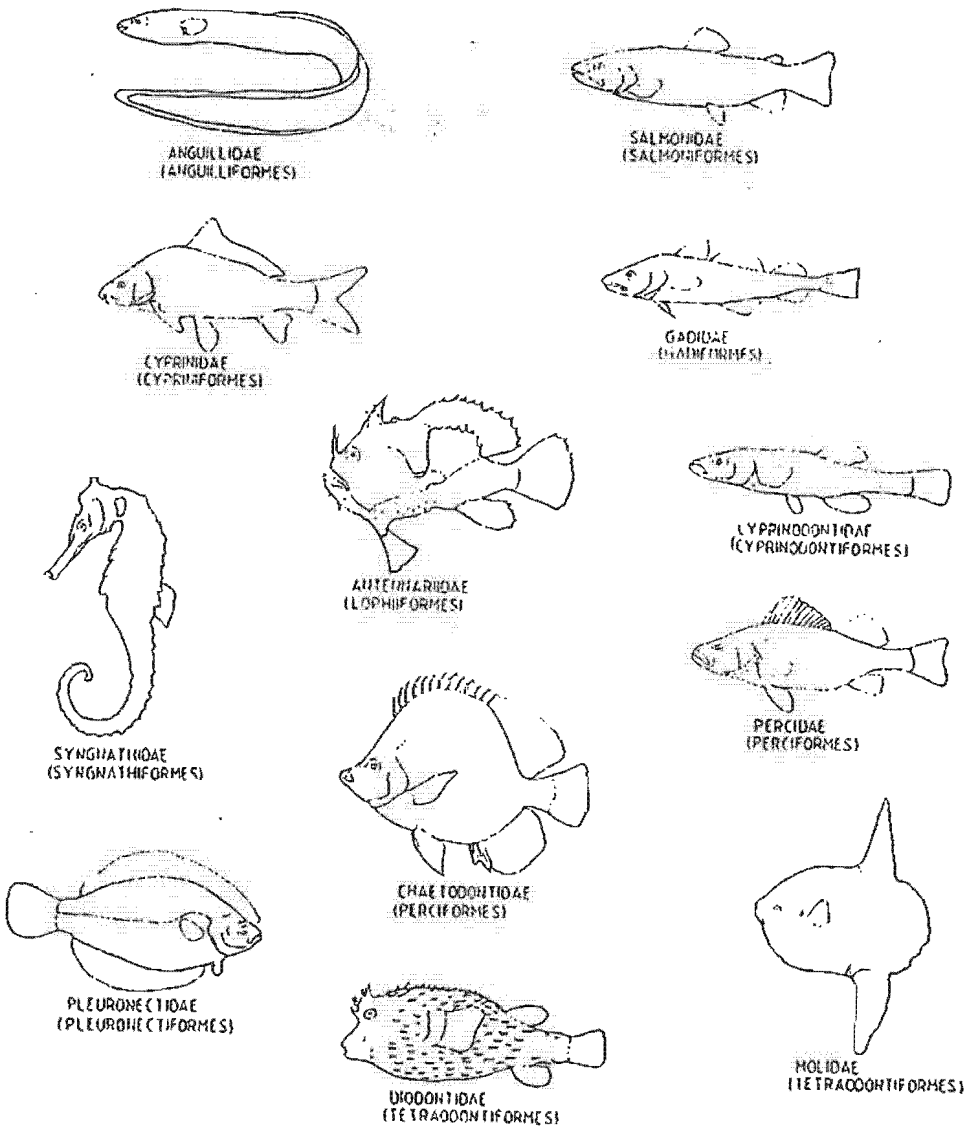


Figure 1. The diversity of teleost form (Lindsey, 1978).

propulsion in water (Bone, 1982). As a medium for locomotion, water has both advantages and disadvantages outlined by Lindsey (1978), which place quite different constraints on aquatic creatures in comparision with their terrestrial counterparts. Water is about 800 times denser and 6500 times more viscous than air, properties which ensure not only that structural morphologies are entirely different from those on land, but that modes of locomotion will be too. The buoyancy of water releases aquatic animals from the limitations imposed by gravity on land; consequently fishes do not require strong internal skeletal structures to support their weight against gravitational forces and need to expend little energy to keep from sinking. Minimizing the constraints of structural weight economics gives scope for the development of extra muscle for streamlining, essential for efficient locomotion through water. Additionally, the presence of a swim bladder in most fish provides buoyancy so that they can suspend themselves at any level in the water column with a minimum of energy expenditure. Of course density also has its disadvantages as it provides opposition to movement: drag is generated by viscous (frictional) and inertial (pressure) forces.

In the course of evolution, fish have developed numerous ways of overcoming the challenges presented by water as a locomotory medium. Their great diversity has ensured the expansion of many variations in the use of body structures and fins for specialised ways of swimming (Wardle and Videlier, 1980). Differences in piscine locomotion reflect differences in the lifestyles and feeding strategies of the fish concerned (Blake, 1983; Boddecke *et al.*, 1959; Gray, 1968).

In all fish however, locomotory propulsive force is provided by co-ordinated muscle contraction and relaxation, and the thrust generated is transmitted to the surrounding medium by the fins (Lindsey, 1978). The various locomotory types

were first described in some detail by Breder (1926), and will be discussed further in the next chapter. The most common form of locomotion in fish however, is by lateral flexion of the tail, or myotome, termed carangiform or caudal locomotion. Other fish swim using specialised paired and unpaired fins and the muscles associated with these (Lindsey, 1978) (Figure 3a).

Whatever the mode of locomotion, two dominant swimming behaviours may be commonly observed in fishes. These have been interpreted by several proponents (Wootton, 1990; Lindsey, 1978; Webb and Weihs, 1978;) as sustained "cruising", and rapid swimming "bursts." Quentin Bone (1966) originally defined the concept of the 2-geared propulsion system, which emphasises the idea that fish have two separate independent motor systems in their myotomes, which are recruited in response to specific demands of velocity.

In order that a fish may exploit it's range of swimming speeds, their locomotory musculature contains a number of differentiated cell (fibre) types which contract at different rates. Goldspink and Johnston (1980, 1981) describe four main types in fish; these are slow twitch oxidative (termed "red"), fast twitch glycolytic (termed "white"), slow twitch oxidative intermediate (pink), and tonic (small diameter) fibres. Each fibre type can be differentiated in terms of contractile properties, metabolism, and cellular structure; therefore they can be identified by means of histochemical and ultrastructural analyses (Johnston *et al.*, 1974). As previously outlined, different muscle fibre types are recruited for different purposes. Fast speed swimming utilises white muscle fibres, whereas slow speed sustained activity is effected by red muscle fibres (Bone, 1966). In addition, many fish have developed intermediate (pink) muscle fibres, which are recruited to enable a smooth transition between the "low" and "high" gear systems (Johnston *et al.*, 1977), although very little work has been done on these fibres (Johnston. 1981; Davison and Goldspink, 1984). Some fish do not possess

any red muscle at all, relying exclusively on the pink fibres for oxidative locomotion (Tulloch, 1990).

The relative proportions of the various muscle fibres are known to be influenced by the mode of life of the fish (Boddecke *et al.*, 1958; Greer-Walker and Pull, 1975). The proportion of oxidative red fibres in the myotome is highest in active pelagic families and lower in less active, bottom dwelling fish and those which primarily use the fins for locomotion (Tulloch, 1990). The present study examines several aspects of the locomotory musculature of one of the most unusual representatives of the latter category, the seahorse *Hippocampus abdominalis* (Figures 2a, 2b).

The characteristics of this graceful creature make it one of the most curious and delightful fish found anywhere in the world. Unique in physical appearance, the long coiled tail and portly body of the seahorse are further complimented by the nature of its locomotion. In particular the tail of the seahorse is highly innovative in that it has relinquished the traditional piscean purpose, employing no discernable locomotory role as it might in a typical fish. Instead, it has become prehensile and remodified as an anchor which allows the animal to attach itself securely to a suitable vantage point while it feeds. It is easy to forget that the bizarre features of this unorthodox creature are no miscarriage of design or construction, but simply an example of the remarkable diversity of modern piscine forms.

The seahorses belong to the order Gasterosteiformes (Teleostei), defined as the fishes with soft rayed fins. This order includes the sticklebacks, flutemouths, bellowsfish, trumpetfish and those members of the family Syngnathidae, the pipefishes and seahorses. Syngnathids are classified as small carnivorous demersal fishes with an external covering of rigid bony plates

Figures 2a, 2b. The seahorse *Hippocampus abdominalis* (Reproduced with permission from Malcolm Francis).

(Francis, 1988). They are most abundant in shallow subtropical and tropical seas, although some are found in temperate waters. The dozen or so seahorse species are all marine. New Zealand has only one species, *Hippocampus abdominalis*, which is found throughout the New Zealand region and also in southwestern Australian seas (Thompson, 1981) (Figure 3b).

The syngnathids are poor swimmers and most more or less drift with the current. Movement is effected by rapid undulation of the dorsal fin or by fanning the pectoral fins. Pipefishes swim in a typically piscean horizontal position, but seahorses differ in that they have adopted an upright stance. A well developed swim bladder is important for buoyancy control and helps to compensate for their limited swimming ability.

Seahorses inhabitat seaweed covered areas in harbours and along sheltered coastlines, from low tide level down to depths of about 50m (Ayling and Cox, 1982). They are usually found resting, anchored securely to seaweeds with their prehensile tails, or swimming slowly near kelp cover. Their diet consists of small crustacea such as amphipods and copepods which are taken from the sea floor or the seaweed. The seahorse captures its prey by approaching slowly and inflating its cheeks and throat in order to suck up the animal. Camouflage plays an important part in this process as the seahorse has no resources of speed to call upon, only the element of surprise. Accordingly, body colouration usually resembles the colour of the seaweed that the seahorse inhabits, varying from brown to greyish yellow through gold, usually with dark spots. Males have head projections which help them to blend in with their surroundings. Females lack this decoration and also tend to be more squat in posture (Thompson, 1981).

The body of the seahorse is laterally compressed with only the dorsal and pectoral fins present. Some species possess a rudimentary anal fin, but it is not found in *Hippocampus abdominalis*. The scales are modified into a series of

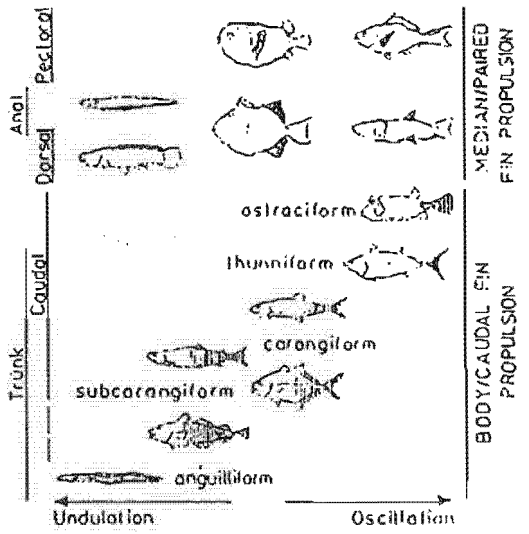


Figure 3a. Modes of locomotion in teleost fishes. (Lindsey, 1978).

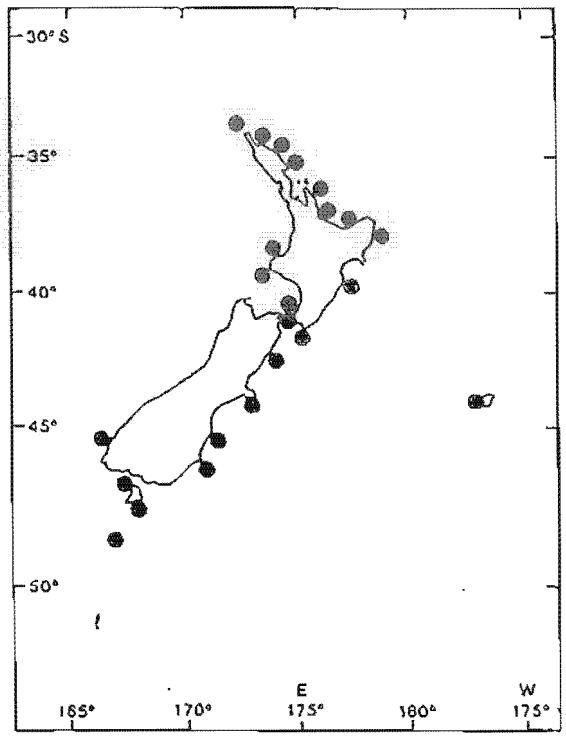


Figure 3b. The New Zealand distribution of *Hippocampus abdominalis*.

(Reproduced with permission from Chris Paulin and Clive Roberts).

protective bony plates which are firmly connected. Teeth are absent, and the gill openings are reduced to a small pore on the dorso-lateral side of the head. Male seahorses possess a brood pouch on either the underside of the tail or on the abdomen, as in the case of *H. abdominalis*.

No information is available on age, growth or maturity factors of the life history of seahorses. However it is known that their spawning usually occurs in the spring, and that the female passes the eggs to the male, which incubates them in the brood pouch until they are ready to be expelled. Fertilisation probably occurs at the time of transfer. The eggs hatch inside the pouch after 30-50 days, following which the young are ejected by the male pressing his abdomen against a hard object and forcing them out a few at a time. The young fish emerge fully developed complete with prehensile tails. At first they swim horizontally, then later take up their characteristic vertical position.

The average length of a seahorse is 100-150 mm, with the maximum length recorded being 325 mm. *Hippocampus abdominalis* ranges from approximately 200-300mm (Thompson, 1981).

The seahorse is an interesting animal to study because of its unique physical characteristics. As the locomotory emphasis has shifted from the myotome to the pectoral and dorsal fins, one may reasonably expect that the myotomal muscles may have been altered as a consequence, especially considering the prehensile nature of the tail. Recent studies have implicated the small diameter fibres in a postural role (Davison, 1983a), so it is possible that they may constitute a significant proportion of the myotome. Furthermore, as this animal typically swims very slowly and does not appear to be capable of rapid bursts of speed, there may be little evidence of slow (oxidative) muscle fibres in the dorsal fin.

To date, virtually no work has been carried out on the tail or locomotory fins of any of the species of seahorses. No studies whatsoever have been recorded concerning the only New Zealand representative (Thompson, 1981).

The aim of this study was to examine and compare the myotomal and locomotory musculature of the New Zealand seahorse *Hippocampus abdominalis*, in relation to its locomotion. Behavioural observations were employed to determine locomotory features, while histochemical, quantitative and ultrastructural analyses were used to determine certain properties of the myotomal and locomotory musculature.

The Locomotion of the Seahorse *Hippocampus abdominalis*

Introduction

Locomotion in teleost fish varies considerably between and within species with age and environment. In order to understand the functional significance of a particular species' locomotory musculature, it is essential to be able to classify its mode of swimming and in doing so, identify the mechanical properties and characteristics of its locomotory type.

In his acclaimed 1926 synthesis "The Locomotion of Fishes", Dr. Charles Breder produced an extensive description of the locomotory categories in different fish groups as well as an analysis of the mechanical principles of fish locomotion. Breder classified the undulatory motions of fish into three types: *anguilliform*-typical of highly flexible fishes capable of bending into more than half a wavelength; *carangiform*-undulations limited mostly to the caudal region, the body bending into less than half a wavelength; and *ostraciiform*-body inflexible, undulation of the caudal fin. The nomenclature anguilliform-carangiform-ostraciiform refers not only to movements in the whole body, but to flexures in the dorsal, anal and pectoral fins, although additional names were also coined for those swimming modes involving the fins. Thus a species which passes undulations along long-based fins can be described in a manner analogous to the anguilliform undulation of the body.

The classification of locomotory types is arranged according to propulsive mode and implies no evolutionary or taxonomic affinities. The rationale for this

is that similar hydrodynamic analyses may be applicable to animals which swim in the same way regardless of respective diverse phyletic origins. In fact functional convergence on one swimming mode by taxonomically remote groups has been frequently noted, for example locomotion is anguilliform both in lampreys and blennies (Lindsey 1978).

These cornerstone principles have remained quite relevant to today's thinking. However, Webb (1973b, 1975); Breder (1926); and Bainbridge (1963) have stressed that these classifications refer to average types within an essentially continuous range of swimming modes and should not be applied too rigorously. This conclusion was drawn as there exists an observable graduation from multi-waved undulation through progressive and eventually exclusive concentration of propulsive movement, and also, individual species of fish may show more than one mode of swimming (Davison, 1983b; Davison and MacDonald, 1985; Starling, 1985). An illustratory example is the surf perch *Cymatogaster aggregata* which usually swims with its pectoral fins (labriform mode) but switches to caudal fin locomotion (carangiform mode) when high speeds are more appropriate (Webb, 1973).

While significant advances have been made in the understanding of some swimming modes, the majority have not progressed beyond Breder's outlines. Following Gray's 1933 classical description of locomotion in the eel, numerous quantitative observations have been made relating body and caudal fin movements to swimming speed, whereas in contrast the more complex locomotory patterns involving paired and non-caudal median fins have been largely avoided. The fact that fish propulsion patterns are so diverse has not influenced the trend that studies of fish locomotion have concentrated on the relatively simple mechanics of body/caudal fin propulsion (Bone, 1989; Davison and Goldspink, 1977; Greer-Walker and Pull, 1975; Blake, 1978; Webb, 1973).

Studies of non-caudal fin propulsion such as the amiiform, gymnotiform, balistiform and tetraodontiform modes of swimming are best understood when reconciled with a basic knowledge of the relevant mechanics. Essentially, these swimming methods evolved with the development of versatile propellors provided by fin rays moving on universal joints. While the fins of the primitive sharks and rays can only be tilted or moved up or down, most teleost fishes possess remarkably flexible and versatile fin musculature, especially those which have reduced their myotomal locomotory musculature. The fins are positioned vertically, and have flexible jointed rays (lepidotrichia) which are supplied with basal muscles that enable the fish to fold the fins, and to make delicate propulsive movements. These intricate fin structures have permitted not only the advancement of innovative locomotory methods, but remarkable diversification of individual patterns of teleost life. Their basic arrangement is worth appreciating in the interests of understanding their structure and complexity.

The flexible web of each fin ray is a double sheet supported by stiff rod-like spokes of a fan. Each rod is attached at its base by a hinge or swivel joint to internal supports buried in the body, and is operated by sets of muscles which can swing it in one or more planes. In most teleosts the pectoral fin base is a row of hourglass-shaped radials or actinosts which are joined at the vertical posterior margin of the scapula and coracoid of the pectoral girdle. Their number is most often four, but may be higher or lower. The radials of teleost pectorals are progressively longer from top to bottom, and therefore when extended the fin tends to swivel about the anteriormost ray as an axis. A nearly vertical hinge for the fin base allows fanning with the pectoral in order to allow a stationary position. Rays in the dorsal and anal fins of most teleosts correspond one-to-one with their internal supports and can be erected, depressed or inclined freely on a universal joint. The entire fin complex is

usually inserted into the fish as a large self-contained unit, without serial correspondence to the adjacent myomeres (Gosline, 1971).

The evolution of these complex fin structures has its origins as far back as the Devonian era, when ray-finned fishes (actinopterygians) first became distinct in the fossil record. Earlier fishes were heavily armoured, with virtually inflexible fins, a feature which gave little scope for efficient swimming or the exploitation of non-caudal locomotory types. Loss of the restrictive armour resulted in reduced acceleration resistance (Webb and Skadsen 1979, Webb 1982c) and enabled the development of flexible and subsequently paired fins. There is no fossil evidence of the origin of fins, although it seems probable that those of the gnathostomes (jawed fishes) arose from lateral dermal folds, seen on certain Upper Silurian anaspids (Moy-Thomas and Miles, 1971). Adaptations in fin and muscle arrangements as well as general structure resulted in enhanced diversity and overall efficiency of swimming modes, including the improvment of non-caudal (transient) swimming performance. The development of a large caudal area enabled increased acceleration in some groups, whereas the appearance of the paired fins conferred the ability for delicate and rapid manoeuvre in others.

Modern fish fins show a great variety of form and function according to the individual design of the fish and its mode of life. The most recent teleosts show optimal structure for slow swimming; the pectoral fins have migrated dorsally from their primitive ventral position, while the pelvics have moved forwards to lie more or less below the pectorals. These locations provide for a wider variety of propulsive and control forces that have been important in penetrating a diversity of habitats (Harris 1937, 1953; Alexander 1967; Gosline 1971, 1980). However it appears that specialisation has been made at some cost to sprint

performance (Hobson and Chess 1978, Webb 1982b).

In contrast to anguilliform or carangiform fish (particularly pelagic species), these specialist swimmers are obliged to spend much of their time foraging at low speeds. Some of the ostraciiforms (the gymnotids and notopterids) are capable of brief bouts of high-speed swimming in the anguilliform mode (Blake 1982b), but typically most specialists are incapable of attaining considerable velocities. These fishes tend to take forms which are unsuitable for high velocity locomotion, whereas fast-swimming fishes are characterised by a streamlined body shape. In being so, they are designed to exploit the use of speed and acceleration to catch prey and escape enemies; a recurrent feature in modern fish.

Nevertheless, the advantages of rapid locomotion are not necessarily essential for the successful interception of food or the evasion of enemies. Many slow swimming coral reef fish do not require speed and rapid acceleration to catch their prey; some are herbivorous, while others feed on small benthic or slow swimming crustaceans (Hobson 1974). Furthermore, swimming at low speeds releases these fish from the morphological constraints associated with a streamlined body form; many display remarkable feats of camouflage which help in the capture of prey, while those that cannot escape predators with high-speed responses are compensated for by the protection of armour or poison. In fact, although some undulatory fin swimmers have retained a typical piscean profile, most are characterised by unstreamlined and frequently eccentric body forms. The Balistidae and Tricanthidae are covered in rough scute-like scales and possess retractable dorsal spines, whereas the Ostraciidae (cowfishes and trunkfishes) are protected by fused bony plates, as are the Syngnathidae, the pipefishes and seahorses. Other families such as the Aleuteridae may not possess armour but are unpalatable to enemies or even poisonous; the boxfish, *Ostracion*

lentiginosum ensures its safety by releasing a highly toxic substance into the water.

The evolution of these protective adaptations has enabled considerable diversification and refinement of undulatory and oscillatory median/paired fin locomotion. Since low-speed swimming accords the opportunity for precise manoeuver, the exploitation of niches unsuitable for fish employing other locomotory modes may be undertaken. Undulatory and oscillatory fin swimmers are typically most competent in manoeuvre, often being capable of turning on their own axis with no lateral translation of the body, or moving backwards almost as well as they do forwards. These capabilities permit the invasion of structurally complex habitats which by their nature require finely tuned control for the efficient utilisation of available resources, such as nutrition and shelter from enemies. In contrast, pelagic fusiform fish designed for efficient steady cruising tend to perform relatively poorly in manoeuvre eg Scombridae (Blake 1976). Accordingly, trends in bony fish evolution pertinent to locomotion involve compromises between optimal designs for slow-speed precise movement and those for faster locomotion.

The trend towards slow swimming and attendant control over manoeuver is attributable not only to the development of versatile fins but also in part to the refinement of buoyancy. Webb (1982) suggested that neutral buoyancy was an essential feature of slow swimming and precise manoeuvre, but many slow-swimming forms capable of fine manoeuvre are negatively buoyant. These fish are obliged to keep swimming in order that sufficient dynamic lift may be generated (Magnuson 1978). In these cases, the lift forces required to counter excess weight over buoyancy are actively generated by the fins. The advantage of negatively buoyant benthic fishes is that frictional forces between the body and the substrate are generated, which aids the fish in holding position

(Alexander, 1966).

While it seems that no hard and fast rules can be applied in this matter, it does appear that the *regulation* of buoyancy is crucial in precise locomotion. Control of both the amount of lift and its distribution along the length of the body imparts the ability to hover and manoeuvre. These features are critical for the avoidance of detection and capture by predators as well as the discovery and interception of prey in many species; therefore, variables such as depth in the water column, attitude and posture must all be monitored. Many non-caudal fin swimmers are adept at controlling the amount and centre of buoyancy, producing particular attitudes so that in appearance and posture they resemble the surrounding vegetation (Sazima and Vieda 1979). *Hippocampus* and its relative *Siphostoma*, the pipefish have developed this feature to the extent where it is pivotal to their mode of life. The inconspicuous attitudes and movements typical of such fish interrelate with the physical camouflage to help ensure successful merging with the environment.

Aspects of reproduction also highlight the importance of buoyancy as a factor in median/paired fin swimming. Attributes such as fertilisation, egg transferral where appropriate, and compensation for per cent weight changes in the body of the fish relative to gravidity and subsequent egg or young expulsion are all dependant on precise control of buoyancy. Therefore, it is not surprising that all undulatory and oscillatory fin median and paired fin swimmers are well accorded in this department; the seahorse is no exception. In every aspect of precisely controlled locomotion, this animal displays considerable accomplishment and is therefore worthy of further investigation.

The purpose of this section of the study was to determine the nature and classification of the locomotion of the seahorse *Hippocampus abdominalis* in relation to its specialised mode of life. General locomotory and behavioural characteristics were observed and recorded, as revealed by simple visual inspection in the aquarium, and by prompting demonstrations of predator evasion techniques.

Preface to Methodology

Body morphology and swimming types place a number of demands on the locomotory musculature, hence it is necessary to observe fish swimming in order to determine the functional significance of their muscle physiology. However, there are two details I would like to point out.

In 1982, Webb and Weihs stated that fish shapes had evolved partly in response to hydrodynamic requirements, eg streamlining for low drag and other modifications. This theory has since been redefined; recent studies have implicated the existence of at least two different functional trends, each leading to a different specialised form: one for sustained (steady state) motion, the other for transient movements.

Descriptive and analytical methods for the examination of various locomotory types may not necessarily be applicable for representatives in both categories. The relevant example in this study was the potential use of a water tunnel (swimming machine), a useful device which enables the determination of fish swimming speeds at different current velocities and the measurement of the critical swimming speed (U-crit). These analyses, which are often employed in locomotory descriptions are only appropriate for steady state locomotory methods where the fish are trained to swim at a constant speed in one direction (Lindsey 1978). Since transient swimming methods are by their nature unsteady, any results obtained from transient swimmers by this method would be unquantifiable and misleading; therefore fish that employ the latter modes of locomotion are entirely unsuitable for experimentation of this type. In accordance with these guidelines, no such investigations were carried out with the seahorse, which is unequivocably a transient swimmer.

Secondly; as the following section of the chapter will endorse, the fins of the seahorse are known to move extremely rapidly, and thus it is difficult to evaluate their movements. This has always posed a problem, in fact until 1942, the forces produced by the vibratory fins serving to propel the seahorse had never been analysed carefully (Breder and Edgerton, 1942). Critics such as Schlesinger (1911) had discussed the locomotion in general terms, but the high speeds at which the fins were vibrated and the consequent difficulties in interpretation were to prove insurmountable in the pursuit of an adequate description. The development of high-speed motion pictures and still photography provided a functional and practical tool with which to effectively access the detailed study of phenomena of this type.

However, in this particular study, it was not felt that such detailed analyses were necessary as the fundamental aims of the thesis were to examine the musculature of the seahorse in *relation* to its locomotion, not the precise mechanics of its mode of swimming.

Materials and Methods

Seahorses (*Hippocampus abdominalis*) are readily available all year round, and are known to survive well in captivity. For the purposes of this study, they were collected by trawl from Lyttleton Harbour, Christchurch. The animals were transferred to the aquarium of the Zoology department at Canterbury University, where they were kept in recirculating seawater at approximately 16°C and fed on brine shrimps. They were determined to have overcome the stress of capture and their new environment if they accepted food, which they did.

The locomotion of *Hippocampus abdominalis* was investigated in the aquaria where the fish were maintained. Initially, the general behaviour and locomotion of the animals was observed as they moved about normally in the tank. Secondly, they were encouraged to swim as if escaping a potentially threatening situation by mild prodding with a blunt instrument. Finally the fish were gently seized and prised free from their substrate holds to determine their reaction to the occurrence of such an event.

The latter two experiments were performed in order that predator escape responses could be noted. Information obtained was recorded and subsequently correlated with data from future sections of this study.

Results

Hippocampus abdominalis is a relatively inactive fish, spending most of its time attached to the seaweed on which it lives using its prehensile tail. The tail was observed to play no discernable propulsive role in swimming, although fairly large myotomal movements do enable the seahorse to change its centre of gravity. It was not observed to function in this way when the animal was actually swimming. The tail can therefore best be described as an anchor which enables the animal to fasten itself securely to an appropriate vantage point.

Seahorses are normally found attached in this way to seaweed fronds, and were not observed to swim about as most "typical" fish are seen to do. Their only significant locomotory activity involved the exchange of stationary locations, except when they were threatened. While attached to the seaweed, they appeared to be concerned only with prey discovery and inception.

If forced to swim as if in response to a predator, the seahorse was incapable of attaining speeds much higher than those achieved in normal translocatory locomotion. Furthermore, they appeared to be incapable of maintaining maximal velocity, often attempting to return to the substrate. When seized, the captive responded by vigorously lashing its tail back and forth in an attempt to escape.

The locomotion of the seahorse was effected by rapidly undulating the pectoral fins and the dorsal fin, the latter providing the thrust for locomotion. The pectoral fins appeared to be more of a balancing aid, as they were only employed exclusively when the fish was anchored to the seaweed; however teleost pectoral fins usually also function as brakes. The dorsal and pectorals were the only fins employed in locomotion as *H. abdominalis* possesses no anal fin, and neither caudal, ventral or pelvic fins are found on any of the seahorses.

During locomotion, waves were passed downward in both the pectoral and dorsal fins, which were seen to move extremely rapidly. The seahorse was also able to move backwards by reversing the direction in which the waves travelled along the dorsal fin (Figure 4). As seen in many undulatory and oscillatory median and paired fin swimmers, this animal was capable of extraordinarily precise and versatile movement, despite the fact that it was incapable of lateral flexion.

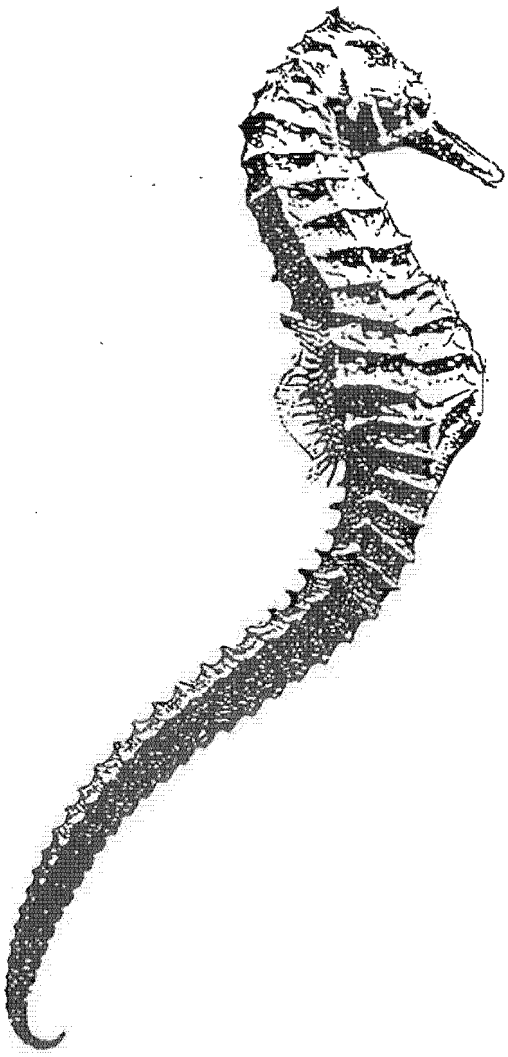


Figure 4. Demonstration of undulatory wave passing down the dorsal fin (Breder and Edgerton, 1942).

Discussion

Observations of the swimming behaviour were simplistic in that factors such as the dorsal fin beat frequency were not measured (this would have involved high-speed cinematography). However, the data obtained can still be used to determine the functional significance of the locomotory and myotomal musculature, and to draw comparisions between *H. abdominalis* and one of its relatives, as well as other species of fish.

As pointed out in an earlier section of this study, Breder (1926) stated that the nomenclature anguilliform-carangiform-ostraciiform could be used to refer to flexures in the dorsal, anal, and pectoral fins as well as the whole body. Harris (1937) has subsequently shown that the characteristic myotomal contractions of a swimming eel-like fish have been preserved in the greatly restricted fin movements analogous to those found in the seahorse. However the locomotion of the seahorse is correctly classified as the *amiiform* mode of swimming, defined once again by Breder (1926) as "short-based dorsal" fin propulsion. Like all exclusively median/paired fin methods of locomotion, amiiform propulsion is characterised by slow, precise manoeuver. Although lacking both the form and the propellor mechanism for speed, the locomotory equipment of such fin swimmers, with its manifold attributes, makes for an easy adjustment of fine spatial relationship. The extent to which this is displayed by the seahorse is a function of its extremely versatile and well-developed locomotory apparatus.

As outlined, the seahorse swims by passing undulatory waves extremely rapidly down the length of its dorsal and pectoral fins, the former providing most of the propulsive force. Breder and Edgerton (1942) found that individual rays

in the dorsal and pectoral fins of the seahorse may oscillate back and forth as fast as 35 times per second, and that undulations in the dorsal fins may have as many as seven advancing "fronts". Differential effects in locomotion (speed, position in the water column) may be attained by altering wave length, amplitude and speed of propogation of waves (which were normally seen to be of the same velocity in both the pectoral and dorsal fins, although obviously this relationship does not always hold, for there is much differential use of the fins in these fishes). Breder and Edgerton also contended that the individual fin rays appeared to move through cycles at speeds approximating those of flying animals of comparable size, although they pointed out that these forms with which the seahorse is being compared do not operate in a medium nearly as dense as water; therefore the muscular effort is naturally much greater.

The highly specialised locomotion of the seahorse is reflected by several modifications from the piscean norm which have enabled the exploitation of its niche in the environment. In fact, the seahorse displays an extraordinary degree of deviation from the "typical" fish profile. Like its relatives the pipefishes, this animal possesses a prehensile tail, but even the pipefishes have retained the caudal fin and a more piscean appearance and lifestyle. Hippocampus has become modified to an even further extent, and has adapted to an essentially inactive mode of life in which the resources of speed and acceleration have virtually been discounted.

The transferral in locomotory emphasis from fast speed to slow has been parallelled by the transfiguration of its physical form. In combination with its rugosity, the body shape of the seahorse forms a distinct impediment to speed; in fact the entire design is practically the antithesis of the ordinary streamlined fish. Rapid velocity is impossible in that no conceivable locomotor equipment

that the energy in the small body of a seahorse could operate would enable it to proceed at any other than a slow speed. Such variation from the typical hydrodynamic blueprint has long been documented as incompatible with high velocity. Breder (1926) stated that there were only a limited number of types that a fast-moving aquatic form may take, and that only variations within narrow limits were possible without a sacrifice of speed. In support of this hypothesis, it may be noted that in groups of fish that are fast swimmers, there is much less variation in functional body form than there are in details not of a locomotory significance. In contrast, lack of speed is associated not with just one non-streamlined form, but with an extensive array of irregular forms which often serve to disrupt the outline of the fish. The unconventional shape and armour of *Hippocampus* is a further example of such blatant disregard for the mechanical interlocking of factors integral in faster fishes.

In a "typical" fish, the elongate fusiform shape functions as an adaptation for reducing energetic costs of motion through water, while the evolved ellipsoidal shape commonly noted in median/paired fin swimmers has a thickness ratio conducive to the reduction of drag (Hess, 1974). Actual fish shapes are thus a compromise between the round cross-section for minimising drag and the deepening of cross-sections for overcoming inertia and for turning; therefore species that spend more time manoeuvering and moving slowly tend to have deep and narrow body sections. These features may be nicely observed in the body of the seahorse, excluding the tail which probably contributes very little drag. In any case the seahorse is designed for a semi-stationary existence in which the economics of streamlining are not very influential.

The unusual form of the seahorse probably evolved as a functional camouflage, once unfettered by the constraints associated with an active

swimming mode of life. Since *Hippocampus* has no resources of speed and acceleration with which to catch its prey or escape its enemies, it is inordinately reliant on maintaining an inconspicuous presence in the successful pursuit of both activities. Although at first glance the body shape of the seahorse might appear noticeable, it is probably unlikely to attract unwelcome attention because of the tendency of predators to pursue familiar patterns of prey shapes and movement. Furthermore, its irregular profile (exaggerated in the male who broods the young) and associated colouration are an effective camouflage, and a capable mimic of the surrounding environment. Seahorses are known to exhibit cryptic behaviour, in which the effectiveness of individual colour camouflage patterns are enhanced by the active selection of appropriate seaweed backgrounds. These attributes effectively break up the physical outline of the seahorse, therefore enabling it to blend convincingly into the environment. In addition, the slow, precise movement of the fish helps to reduce the chances of its presence attracting the attention of a predator. Even the versatile eyes are designed to minimise the necessary movements made; they are extremely maneuverable and are capable of independent operation.

The evolution of the mode of locomotion of the seahorse is thought to have developed in parallel with the protective armour and prehensile tail. The external carapace provides compensation for the inefficient swimming method in two principal ways; firstly, it assists in camouflaging the fish, but should it be detected, it minimises the danger posed by the majority of enemies. Only those assaillants with sufficiently heavy jaws are able to shatter the tough bony cuirass, and even so, predators with previous gourmet experience of seahorses might well choose to look elsewhere for a more profitable or palatable investment.

This armour does have its detriments, although the seahorse has evolved

ways of surmounting them. It effectively prevents the lateral flexion critical to the locomotion of most fishes; but as the seahorse has such well-developed precision movement, its manoeuvering skills often far surpass those of fast-speed swimmers, enabling it to invade structurally complex habitats. More consequentially, these modified scales are disadvantageous not only in that the benefits of streamlining are lost, but that it is heavy; thus acceleration resistance is increased and structural weight economics are perturbed. These characteristics are potentially detrimental for the seahorse; however because it is fundamentally designed for a largely benthic, inactive mode of life in which speed and acceleration are not required, the importance of acceleratory assistance is minimal. Furthermore, the prehensile tail effectively overcomes the problem of accentuated gravitational forces by providing a means for the animal to anchor itself and forage at any level in the water column without the ongoing maintenance of considerable energy expenditure.

The prehensile tail of the seahorses and pipefishes is virtually unique among fish. In acting as a mooring, it prevents them from being swept away by the current, a valuable attribute in animals which tend to drift with the tide rather than waste energy attempting to swim ineffectively against it. In fish such as these of which cryptic behaviour is a feature, displacement from the selected camouflaged background is undesirable as it reveals the presence of the animal to predators; therefore the prehensile tail is critical to the maintenance of concealment. As well as acting as an effective defense against tidal displacement, the tail also serves to confound attackers attempting to pry it free, and as it is capable of lashing violently back and forth, it functions as a deterrant should its grip be overcome. Large movements of the tail are also employed to change the centre of gravity of the animal as it hovers, and thus enhance its

manoeuverability.

In conclusion, the locomotory method of the seahorse is thought to be accountable for the unorthodox appearance and mode of life of this strange creature. It is also hypothesised to be responsible for the small size of all the members of its family. For mechanical reasons, rapid vibration of locomotor parts in either terrestrial or aquatic animals is limited to small creatures such as hummingbirds and insects. The weight-length ratio controls the power necessary to move a given surface, increasing the required mass of muscle tissue to impossible proportions as absolute size reaches a certain limiting value. Another factor in this restriction of size is that like any other faunal community, the seahorse environment is restricted in dimensions by the circulation of water as well as competition for solar radiation (nutrients). This hypothesis is supported by the distribution patterns of the largest seahorse species which are found in regions populated by giant kelps, and the smallest, which live in part among the small floating clumps of *Sargassum* in the Atlantic ocean (Breder and Edgerton, 1942).

The Locomotory and Myotomal Musculature

Introduction

The great majority of teleosts propel themselves by a combination of lateral movements of the caudal fin and the backward passage of transverse waves along the body (Lindsey, 1978). These processes result from the operation of the axial musculature, built around the flexible notochord archetypal of all chordates (Bone, 1989).

The axial musculature is divided up into myotomal units by connective tissue partitions. Contraction of some or all of the myotomes on one side of the body results in the lateral movement characteristic of carangiform locomotion (Wainwright, 1983). In fish such as the seahorse which do not employ lateral movements of the myotome in locomotion, the relevant fins are developed into locomotory units equivalent to the caudal fin (Lindsey, 1978). Instead of myomeres contracting to bend the body, muscles inserted into the sides of the fin rays contract to deflect the ray in an appropriate direction, and propel the fish in the direction it wishes to travel (Lindsey, 1978).

The organisation of teleost and elasmobranch locomotory musculature is complex. Elementally, the myotomes form a series of overlapping cones, and the muscle fibres in each myotome insert into a connective tissue sheath or myocomma (Bone, 1989). The orientation of fibres within the myotome varies both along the body and with distance from the vertebral column (Alexander, 1969). In general,

superficial fibres run parallel to the surface whereas deeper fibres make angles of up to forty degrees with the long axis of the body, and may be arranged in a spiral pattern. The significance of this arrangement is thought to enable each muscle fibre within the myotome to contract at the same speed, and to allow similar degrees of sarcomere shortening at different body flexures. This enables optimal overlap between thick and thin filaments allowing the generation of maximum tension at all depths within the myotome, and hence optimum efficiency of operation (Bone, 1989). The complex folding of the myotomes is also thought to enable optimum fibre packing in a wide-bodied fish (Alexander, 1969).

Fish myotomes show a phylogenetic increase in complexity from amphioxus to teleosts. According to Bone (1982), this trend is parallelled in individual teleost ontogeny, where the myotomes form first as V-shaped blocks before folding further to give the adult W shape. However, despite the fact that axial musculature organisation and fibre orientation patterns are hardly unsophisticated, this tendency towards complexity is not reflected in patterns of fibre distribution. The heterogeneous mixture of different fibre types seen in most terrestrial vertebrate locomotory muscles is rarely observed in teleost fish, in which a simple anatomical separation of fibre types can usually be observed within the myotome (Bone, 1989). According to Dana Ono and Kaufman (1983), the evolutionary replacement of the lower vertebrate zoning pattern by the higher terrestrial vertebrate mosaic matrix is directly related to the effects of gravity, which restricts the development of large blocks of a single muscle type that is only for occasional use.

Profiles of muscle fibre types do, however, vary in accordance with trophic demands. Dana Ono and Kaufman (1983) recognise four main fibre distribution groups: single fibre, zoned, mosaic and zoned-mosaic. The occurrence of mosaic

patterns in some fish branchial muscles is usually associated with generalist feeding patterns, a fact which suggests that mosaic muscles are initially single fibre type muscles exposed to complex functional demands, whereas the typical zoned configuration reflects specific functional purposes for different muscle types (Dana Ono and Kaufman, 1983). Accordingly, the pattern and number of fibre types present in a given functional muscle unit varies depending on the role it is expected to play.

Bone (1966) surveyed axial muscle patterns in protochordates and fish and suggests that originally axial muscle fibres were of a single type. They are thought to have formed a series of muscle cells along the length of the body, which were later organised into serial myotomes electronically separate from each other. With evolutionary increases in physical size, and the diversification of locomotory structures, myoseptal development and serial activation of the axial muscle fibres along the body gradually became implemented. These innovations effectively transformed the system into separate myoseptal units in which the major fibre types became specialised in different directions. From this point, further divergence from the basic two categories has occurred.

The number of distinct teleost fibre types varies from two to five and varies from species to species, depending on mode of life (Bone, 1989). A few fish, such as the electric fish *Eigenmania virescens*, which relies mainly on anal fin mediated locomotion, have only a single muscle fibre type (white) in its trunk muscle (Behrend, 1986). Nonetheless, most fish have at least two basic types in their axial musculature. These fibres operate on different fuels utilising different routes for ATP production, and are commonly described by their characteristic colours, red and white. However they are most correctly defined in terms of their contractile

nature.

Electromyograms have established that the red portion of the myotome is active when the fish swims slowly, while under these cruising conditions no electrical activity is seen in the white fibres (Johnson *et al.*, 1977). The white muscle is employed during bursts of rapid transient activity. It operates by the propagation of action potentials, and typically contracts rapidly, hence the term 'fast' fibres (Bone, *et al*, 1978; Johnston *et al*, 1977; Kilarski *et al*, 1982). In contrast, no action potentials have ever been observed in the 'slow' red fibres, which are driven by long-lasting contractions evoked by depolarising agents (Davison, 1983a; Johnston *et al*, 1977). Differences in contractile profiles are important in determining not only the specific role of the fibre types in locomotion, but the physiology of the fibres themselves.

Contraction of the musculature is thought to be best described by the sliding filament hypothesis, which involves increased overlap between adjacent filaments (Hill and Wyse, 1989). Energy stored within the bonds of the molecules adenosine triphosphate (ATP) and phosphocreatine (a storage form of high energy phosphate groups), originates from the breakdown of fatty acids, glucose or glycogen, depending on the pathway employed. The activity of the protein catalyst creatine kinase, the main protein found in the region of the M-line, stimulates the transfer of a phosphate group from phosphocreatine to adenosine diphosphate. This operation provides the power needed for contraction. The energy yielded produces a movement of the myosin head; thus actin is pulled past the myosin filament and the thin filament is drawn further into the A band. Consequently, sarcomeres shorten during each contraction (Hill and Wyse, 1989).

Muscle contraction depends on the availability of calcium ions, which is

regulated by the sarcoplasmic reticulum, a well developed membrane system consisting of segments of anastomosing tubules surrounding each myofibril. Near the junction of what is usually found in teleosts to be the z-line, the tubules become confluent, forming flattened sac-like structures called terminal cisternae, which are associated with another membrane system, the transverse tubules. Each tubule in the T-system originates as an invagination of the sarcolemma, and forms a grid-like system that surrounds each myofibril at the z-line. The whole unit is collectively known as a triad. Depolarisation of the sarcolemma is initiated at a specialised myoneural junction on the surface of the muscle cell, and is transmitted to the sarcoplasmic reticulum at the point at which the triad is situated by the enzymes of betaoxidation, located in the mitochondrial matrix.

When depolarisation of the sarcolemma occurs, an inward spread of excitation extends to each myofibril at the point of the z-line, effecting the release of calcium ions from the terminal cisternae into the vicinity of the thick and thin filaments. Here, they bind to troponin, a protein in the filaments, and allow bridging of the actin and myosin filaments. When the membrane depolarisation ends, the sarcoplasmic reticulum acts as a sink for calcium ions, which it actively transports back into the cisternae, ending contraction (Hill and Wyse, 1989).

Variation in the force of contraction is controlled by the recruitment of different numbers of muscle fibres (Lindsey, 1978; Bone, 1989). Since white muscle fibres are typically present in large numbers, potential fibre recruitment is high, and hence the force of contraction may be considerable. In contrast, the oxidative musculature is limited in terms of expansive development. It must be supplied with oxygen, the availability of which is limited by the surface area of the gills. Consequently, potential fibre recruitment is relatively lower than that of the white

muscle, and forces of contraction are less significant (Johnston *et al.*, 1977).

The success of this bimodal locomotory structure is reflected by the extensive exploitation of a wide range of power outputs from the axial muscles, over even modest speed ranges. The universal distinction between slow red aerobic fibres employed in slow sustained cruising, and the fast speed anaerobic fibres used in burst swimming indicates not only that this system is well established among teleost fish, but that it must have appeared early in chordate evolution (Bone, 1989).

The differentiation of muscle into the various fibre types is determined by the specific type of innervation (Lindsey, 1978; Bone, 1989). Red muscle fibres and fin muscles in all fish possess a distributed cholinergic pattern of innervation defined as endplates all along the muscle fibres fibre (Bergman, 1964; Bone and Ono, 1982). White muscle fibres exhibit either multiple or more usually terminal (end plates only on the myoseptal ends of the muscle fibres) patterns of innervation (Barets, 1961; Bone and Ono, 1982; Bone, 1989). Bone and Ono's extensive survey of 125 fish families (1982) has revealed that virtually all families display either the primitive or the derived form of innervation, although some orders such as the Osteoglossiformes and Ostariophysi contain families displaying both types. The terminal pattern is believed to be the primitive form, while the multiple innervation is thought to be derived. Bone (1970) has suggested that the type of innervation may even serve as a taxonomic character, as none of the more modern acanthopterygians have focal innervation, and of the orders that are considered primitive such as the Salmoniformes, only the Salmonidae are multi-terminally innervated (Bone, 1970).

In addition to differences in patterns of innervation and function, red and white fibres show structural and metabolic differences, which are evident from the time

of their formation (Bone, 1978; van Raamsdonk *et al.*, 1982). The fast contracting (white) fibres operate by anaerobic glycolysis (Bone *et al.*, 1978; Johnston *et al.*, 1977; Kilarski *et al*, 1982); a feature which is reflected in all facets of their character. As they do not utilise an oxidative energy pathway, their vascularisation is relatively insignificant; there is little evidence of myoglobin or of oxidative enzyme activity, and they have small, sparsely distributed mitochondria.

Activity levels of the enzymes characteristic of anaerobic glycolysis are higher in white fibres than those observed in red fibres. The fact that anaerobiosis is not dependent on short oxygen diffusion distances eliminates the limitations imposed on dimensional development to a certain extent. Correspondingly, the diameters of white fibres are large; sometimes more than 300 μm; and furthermore, they vary considerably in this regard.

White muscle typically constitutes the bulk of the myotome. This feature is consequential not only of the substantial relative size of these fibres, but of the fact that it is functionally necessary for them to be present in large numbers (Lindsey, 1978). Although the fast fibres produce a greater power output than the slow-contracting red musculature (Johnston *et al.*, 1977), the operation of anaerobic glycolysis invariably results in an oxygen debt and the subsequent accumulation of lactate. Consequently, rapid fatigue of the white muscle is inevitable, and while it provides the force required for rapid bursts of speed, its operation is unsustainable (Bone, *et al.*, 1978; Johnston *et al.*, 1977; Kilarski *et al.*, 1982).

In contrast, the slow-contracting red fibres do operate at a sustainable level. They function aerobically and are therefore characterised by an oxidative enzyme system (Bone *et al.*, 1978; Johnston *et al.*, 1977; Johnston *et al.*, 1981). Packed with mitochondria and blood vessels, the oxidative capacity of these fibres is extensive

in order to enable the utilisation of aerobic lipolysis or glycolysis as a means to obtain ATP (energy).

The slow fibres typically comprise a major proportion of the fin muscles and a relatively minor component of the myotomal body muscle (Davison and McDonald, 1985; Johnston, 1983). They are found as a thin superficial strip (and in some species, notably tunas, a deep internalised strip), which constitutes between 0.5 and 29 per cent of the total muscle, depending on species and mode of life (Greer-Walker and Pull, 1975). Red fibre proportions are typically highest in active pelagic families and lower in less active, bottom dwelling fish and those which primarily use their fins for locomotion (Boddecke *et al.*, 1958; Greer-Walker and Pull, 1975; Mosse and Hudson, 1977). Furthermore, fish from within the same species develop more red muscle if they are exercised (Bone, 1978; Davie *et al.*, 1986).

In the myotome, red fibres run parallel to the long axis of the fish in a peripheral wedge adjacent to the lateral midline (Kryvi and Totland, 1978; Patterson *et al.*, 1975). According to Love (1970), red muscle fibres contain more haem pigment, cytochrome c, glycogen and lipid, but less protein and water. They are usually uniform in morphological design; typically conforming to a regular shape and small size (only 20-50 percent of fast fibres), in order to enable the efficient diffusion of oxygen. These properties reflect the capacity of red fibres for supporting slow speed sustained swimming aerobically (Patterson and Goldspink, 1972).

Histochemical, ultrastructural, electrophysiological and biochemical studies all support the view that the the fast fibres are specialised for anaerobic glycolysis during short bursts of rapid swimming, while the slow fibres are designed for sustained aerobic operation during cruise swimming (Bone, 1989; Bone *et al.*, 1978;

Johnston *et al.*, 1977;). There seems no reason to doubt that the duality of function in gnathostome axial muscle is archetypal of chordate phylogeny; however there are certain aspects of the overall picture which question this concept and are worthy of further consideration. In particular, the origin and functions of the other fibre types remains a topic of debate.

Many teleosts possess a transitional zone of intermediate (pink) fibres, sandwiched between the outer slow red fibres and the inner fast white fibres, which appear to be intermediate in mitochondrial and enzyme content (Johnston, 1980; Scapolo and Rowlerson, 1987). Electromyographic studies indicate that they appear to be recruited at swimming speeds intermediate between slow cruising and maximum burst speed (Davison and Goldspink, 1984; Johnston *et al.*, 1977). These results, together with the fact that they are usually positioned between the red and white muscle groups (Johnston, 1980; Scapolo and Rowlerson, 1987), have inspired speculation that the pink muscle enables a smooth transition between the 'low' and 'high' gear systems (Davison and Goldspink, 1984; Johnston *et al.*, 1977).

The fact that not all teleosts possess red muscle, and therefore rely exclusively on pink fibres for aerobic locomotion (Tulloch, 1990; Mosse and Hudson, 1977), tends to complicate the issue. Recent studies have demonstrated that pink fibres display considerable variation in morphological and chemical profiles which are undoubtedly related to interspecific functional requirements (Mosse and Hudson, 1977). Furthermore, pink muscle may be present in more than one population in the musculature (Tulloch, 1990; Akster, 1985; Mosse and Hudson, 1977). These different fibre populations are probably recruited in response to different locomotory requirements.

The intermediate fibres are not the only fast contracting fibres to have been

implicated in other than the archetypically defined role. While the 'fast' and 'slow' motor systems are certainly functionally separate in sharks, lungfish, lampreys, *Amia*, amphioxus, and some primitive teleosts, such as the eels; according to Hudson (1973), Johnston, Davison and Goldspink (1977) and Bone, (1978), both red and white fibres of certain species are recruited at *sustainable* swimming speeds. In fact; in some fish, certain muscle fibres have developed functions which have little to do with locomotion, except that mode of swimming has facilitated its development. Some members of the Scombridae such as the skipjack tuna have internalised red muscle masses; a feature associated with counter-current vascular heat exchange. This exceptional musculature enables elevated red muscle and brain temperatures to be maintained over a wide range of ambient temperatures. The presence of such fibres in the myotome is an adaptation to the extremely active lifestyle of these pelagic fishes (Bone, 1978b).

A non-locomotory function for the superficial red fibres of some teleosts has also been suggested. It has been proposed that the red muscle in fact functions as a liver, taking catabolites from white muscle and exchanging them for fuel (Braekkan, 1956). While there certainly appears to be irrefutable evidence that red muscle has a contractile function, evidence to the contrary has prompted many workers to resist reducing the status of the theory to mere speculation, and the idea has remained persistently controversial.

Further muscle fibre types have also been reported, depending on the investigative technique employed and individual fish species. In some fish, there are muscle fibres which are characterised by the fact that their dimensions are the smallest of all the fibres. Logically, they are known as 'small diameter', or tonic

fibres (Korneliussen *et al.*, 1978; van Raamsdonk *et al.*, 1980; te Kronnie *et al.*, 1983; Kilarski and Koslowska, 1985). These fibres typically have relatively few mitochondria, and their rates of contraction are thought to be very slow (Kilarski and Koslowska, 1985). Other tonic fibres with similar properties but a much larger diameter have also been described in several freshwater teleosts, such as *Gasterosteus* (Kilarski and Koslowska, 1983).

The tonic fibres have been subject to relatively little investigation. It is hard to establish what role they play, although they have been compared to the postural fibres of mammals, and may function in a similar way in teleost fish (Davison and McDonald, 1985; Johnston, 1981; te Kronnie *et al.*, 1983; Morgan and Proske, 1984). In view of their contractile nature, morphology and typically low proportions in the musculature, the suggestion that they play any role in locomotion must be questioned.

The object of this section of the thesis was to determine the fibre types present in the myotomal and locomotory musculature of the seahorse *Hippocampus abdominalis*, and to explain the significance of the results obtained. The individual fibre types have different metabolic and contractile properties which enable them to be identified on the basis of their histochemical staining for metabolite stores and enzyme activities. The fibre types also exhibit distinct morphological and fine-structural profiles which may be observed when viewed by a transmission electron microscope, and which provide a further means with which to identify the muscle types present.

In terms of histochemistry, red fibres stain intensely for glycogen and lipid, but

stain very lightly for myofibrillar ATPase activity as their enzymes are 'unstable'-the first to be deactivated by the myofibrillar ATPase preincubation technique (Bone, 1978; Kryvi and Totland, 1978).

In contrast, the white fibres have low glycogen and lipid stores and stain for a predominantly anaerobic type of metabolism, characterised by high glycolytic enzyme activity and low oxidative enzyme activity (Johnston, 1980, 1981). These fibres are mATPase 'stable'; the enzymes withstanding denaturation over a range of preincubation times and pH levels.

Pink fibres are typically intermediate in their staining characteristics for lipid, glycogen and oxidative enzyme activities, although they are the most stable of the fibre types when stained for mATPase activity (Johnston *et al.*, 1977; Mosse and Hudson, 1977).

Small diameter (tonic) fibres show little staining for glycogen and low oxidative enzyme activity (Kilarski, 1990; Davison (1983). According to Johnston *et al.* (1974), tonic fibres display stable mATPase activity; however, Kilarski (1990) and Davison and MacDonald (1985) found that in their study animals, they did not.

Although histochemical techniques provide a useful indication of the fibre types present, the possibility of reaching a false conclusion cannot be discounted (Eichelberg, 1976). Ultrastructural examination of the muscle fibres is therefore essential in order to confirm or deny the data obtained from the histochemical tests.

Furthermore, ultrastructural analysis provides a more detailed comparison of different types of muscle fibres. Ultrastructural criteria for distinguishing between fibre types include numbers and qualities of mitochondria, structure and arrangement of myofibrils, and development of the sarcoplasmic reticulum. Other

features such as the presence of a thicker Z-line in white fibres compared to red (Patterson and Goldspink, 1972), and differences in the deposition of stored metabolites (Bone, 1978a), aid in the identification process.

Under the electron microscope, white muscle fibres are easily detected because of their large size, lack of mitochondria, and rows of regularly packed myofibrils (Patterson and Goldspink, 1972). In contrast, red fibres are characterised by high mitochondrial volumes and irregular packing of the myofibrils (Johnston, 1985). Pink fibres are intermediate in terms of mitochondrial volumes and myofibrillar packing (Davison, 1983). Small diameter fibres have not been thoroughly examined, but to date, studies have shown that they contain few mitochondria, and a high degree of myofibrillar packing (Bone, 1978; Walesby and Johnston 1980; Davison 1983). Furthermore, they have a poorly developed sarcoplasmic reticulum, a feature which is not reflected by the other three fibre types.

In addition to investigative techniques for identifying and describing the fibre types present, quantitative analyses are also useful in that they enable further comparision. Fibre diameters, proportions of individual fibre types, and proportions of cell components (mitochondria, myofibrils, and cytoplasm) were all investigated in the present study.

The results obtained from this section of the study enable a conclusive overview of the locomotory and myotomal musculature of the seahorse to be made, which may subsequently be related to (and explained by) the locomotory characteristics and mode of life of the animal.

Methods

Histochemistry

Fish were captured and maintained as described previously. They were killed by overanaesthetisation in 1% benzocaine in seawater.

Blocks of muscle were cut from the tail at the distal tip, and at approximately the mid point. Blocks were also cut from the dorsal fin musculature (Figure 5).

Tissue was immediately frozen in liquid nitrogen (-178°C), and mounted on a cryostat chuck using O.C.T. compound (Lab-Tek products Inc., U.S.A.). Blocks were gradually warmed to -26°C, then sectioned (10 um) in a cryostat. Sections were mounted on coverslips and stored at -26°C until they were stained. They were subsequently mounted in glycerine jelly on glass slides within one week of production.

Sections were stained for lipid using Sudan Black B. Twenty minutes were allowed for the tissue to incubate. Sections were washed in 50% ethyl alcohol, then mounted.

Sections were stained for gylcogen using the periodic acid Schiff's method (Pearse, 1960).

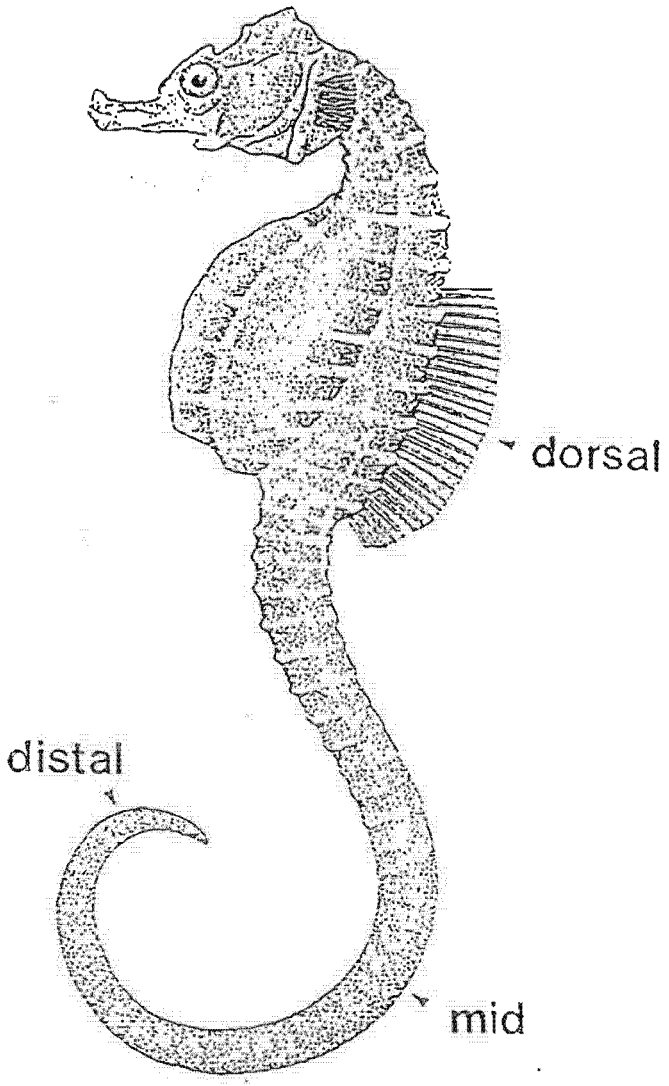


Figure 5. Diagram of *Hippocampus abdominalis* showing the points at which tissue was dissected from: The dorsal fin, the mid tail region, and the distal tail region. (Reproduced with permission from Chris Paulin).

The activity of oxidative enzymes in the muscle cells was visualised using lactate as a substrate and nitro-blue tetrazolium (NBT) as the stain (Nachlas *et al.*, 1958). This reaction involves non-specific NADH diaphorases in the mitochondria breaking down lactate to pyruvate and reducing NAD to NADH. Sites of this transformation are labelled purple by formazan, an insoluble salt formed from nitro-blue tetrazolium (Pearse, 1972). Sections were incubated for 20 minutes at room temperature, then fixed in 2% formalin and mounted.

The activity of myofibrillar adenosine triphosphatase (mATPase) was demonstrated using a modification (Johnston *et al.*, 1974) of the Guth and Samaha technique (1970). The process involves an alkali preincubation (18 mmol/l calcium chloride, pH 10.0, 100 mmol/l Sigma 221 buffer) for 5, 10, 15, or 20 minutes to sequentially deactivate the mATPase enzymes from different muscle fibres with different physiological pH tolerances. The seahorse muscle was extremely stable, so to differentiate between the different fibre types, sections were preincubated at pH 10.4 and 10.5. Sections were then incubated for 20 minutes in a 2.7 mmol/l ATP solution at pH 9.4 (18 mmol/l calcium chloride, 100 mmol/l Sigma 221 buffer). Sites of mATPase activity were visualised by washing the sections in solutions of 1% W/V calcium chloride; 2% W/V cobalt chloride; 100 mmol/l Sigma 221 buffer (pH 9.4); and finally 1% ammonium sulphide. Sections were washed in tap water, then mounted.

The myofibrillar ATPase stain relies on the differing abilities of the myosin enzymes from each fibre type to withstand denaturation of extreme pH, thus it is an artifact stain (Davison and MacDonald, 1985). Guth (1973) demonstrated that the hydrolysis of ATP in the histochemical reaction can result from ATP-ase activity of myofibrils, mitochondria or both and therefore cannot be used to infer that individual muscle fibres are fast or slow depending on the intensity of

staining of the myofibrils. However, Goldspink (1983) showed that myosin extracted from slow and fast fibres had different pH sensitivities, a finding which imparted some validity. Johnston *et al.* (1975) have shown that interference from non-myofibrillar ATP-ases is insignificant.

Fish muscle is more sensitive than mammalian muscle to pH treatment (Guth and Samaha, 1969), thus preincubation pH levels are of paramount importance. A certain amount of interspecific variability exists in the sensitivity of fibres to inactivation. Mosse and Hudson (1977) found that preincubation at pH 10.4 for short periods of time usually 70-80 seconds caused inactivation of most of the red fibres. In most fish, long periods of preincubation causes general inactivation of all but pink fibres.

Guth and Samaha (1972) showed that preincubation at pH 4.35 demonstrates the presence of intermyofibrillar ATP-ases, whilst preincubation at pH 10.4 is relatively specific for actomyosin ATP-ases. This was found to be a good pH for demonstrating the denaturation characteristics of the seahorse muscle.

The work of Guth and Samaha (who originally devised the method!) has demonstrated that the mATPase stain cannot be used as anything but a guide to be exercised with caution (1972). An example illustrating this point is that of the slow-contracting muscles of newborn animals, which stain intensely for mATPase activity due to the stability of their ATP-ases; a reversal of the 'typical' trend. This characteristic is lost in certain of their fibres soon after birth (Guth and Samaha, 1972).

Although concerns have been voiced regarding the reliability of this stain, it is generally agreed that if the technique is used in conjunction with other stains, particularly succinic dehydrogenase, some indication of the nature of the myofibrils can be gained.

Ultrastructure

Small pieces of tissue were dissected from the fish as above (see Figure 5), and immersed in 2.5% gluteraldehyde in 0.1M cacodylate buffer pH 7.4. The tissue was left in this solution overnight, then washed in 0.2M cacodylate buffer pH 7.4, and later postfixed in osmium tetroxide.

Postfixed tissue was dehydrated in an alcohol series, then embedded in Spurr's low viscosity embedding medium. Semithin (1μm) sections were cut on an LKB 8800 ultramicrotome and stained with toluidine blue. These sections were examined under an optical microscope for fibre size and orientation (i.e. longitudinal or transverse). After examination, ultrathin sections (40-60 nm) were cut and mounted on uncoated copper grids. The ultrathin sections were double stained using an aqueous solution of uranyl acetate (30 mins) followed by saturated lead citrate (20 mins). After washing the sections, they were examined under a JEOL 1200EX electron microscope.

Proportions of different fibre types

Histochemical slides of muscle cross-sections were obtained from all three sites as described (Figure 5). Dorsal fin and myotomal cross-sections were stained with the myofibrillar adenosine triphosphate (mATPase) and oxidative enzyme activity (NBT) stains to highlight the oxidative, intermediate and glycolytic musculature. The slides were then mounted and photographed, and a transparent grid was placed over the enlargement to enable determination of the areas occupied by each fibre type.

The proportions of the different muscle fibre types were determined as percentages of the total musculature for the three areas examined: the dorsal fin, the mid tail region, and the distal tail region. Five fish were evaluated for each of three size groups: 22 ± 0.5 cms, 24 ± 0.5 cms, and 29 ± 0.5 cms.

An ANOVA (analysis of variance) test was carried out on the resultant data to determine whether observable differences between the proportions of fibre types in the dorsal fin, myotome and distal tail region were significant ($p<0.05$). The same test was also performed in order to determine if changes in the fibre proportions with increasing fish size were statistically significant.

Proportions of cell components in different fibre types

The proportions of myofibrils, cytoplasm and mitochondria within the muscle cells were calculated from ultrastructural photographs, using a grid of dots superimposed on a transparent sheet and fitted over each picture. Thirty photographs of a reasonably representative stock of each fibre type were measured, with the exception of the dorsal small diameter fibres and inner white fibres, which were not observed in sufficently high numbers to enable accurate analyses to be made.

ANOVA tests were carried out to determine if the differences between the percentages of cell components in the individual fibre types were statistically significant.

Fibre Diameters

Sections stained for mATPase activity were used to determine the diameters of each type of muscle fibre in the dorsal fin and the myotome (Figure 5). At least fifty of each fibre type were measured from the distal tail, mid tail and dorsal sections, using a random transect to determine which fibres from each population to measure. Measurements were obtained with an eye-piece micrometer. The length of the fish from which the sections were taken ranged from 19-29 cm.

An ANOVA test was carried out on the resultant data to determine whether observable differences between the diameters of the different fibre types were significant ($p<0.05$); and whether a significant difference between individual fibre types in fish of increasing length could be demonstrated.

Results

Fibre types present in the dorsal fin

The dorsal fin of the seahorse is a delicate structure. The fin rays insert through openings in the external bony armour deep into the muscles which operate it.

The fibre distribution pattern observed in the dorsal fin musculature appeared to be quite complex. Four fibre types were observed, not all of which were present in homogeneous populations. Of the types which were also seen in the myotome, not all appeared to correspond directly with those present in the dorsal fin.

Adjacent to the dorsal fin rays, a large population of mATPase stable fibres was observed (Figure 12), which occupied a substantial proportion of the dorsal musculature. These fibres stained strongly for oxidative enzyme activity (Figure 6), glycogen (Figures 10a, 10b, 10c), and lipid (Figures 14c, 14d).

From the results of these histochemical tests, these fibres did not appear to constitute an entirely homogeneous population, as a gradual increase in fibre size from the region of the fin rays toward the periphery of the locomotory musculature was observed (Figures 6, 10a). This dimensional trend was parallelled by a decrease in levels of oxidative enzyme activity (Figure 6), glycogen (Figure 10a), and lipid. However, the result from the mATPase typing confirms that these fibres may be specified as a single fibre type, defined as oxidative pink muscle.

The mATPase stain also defined a further type of pink muscle. At regular

points in the oxidative pink muscle fibres, small blocks of larger fibres were observed which stained lightly for lipid (Figures 14c, 14d), glycogen (Figures 10a, 10b, 11a) and oxidative enzymes (Figure 6); and which were mATPase labile (Figure 12). These fibres were also present in a larger block between the dorsal oxidative fibres and a further population of (white) muscle fibres (see below). They were defined as pink intermediate fibres.

White fibres in the dorsal fin were also present in two populations. A substantial peripheral population was observed; also a smaller group which were located adjacent to the dorsal fin rays. These groups were defined primarily in terms of size, but also in terms of proportions and position in the musculature. In contrast, their staining characteristics were identical. They were mATPase stable (Figure 12), and did not stain for glycogen (Figures 10b, 11b), lipids (Figure 14d), or oxidative enzymes; (refer Figure 9a: myotomal and dorsal white fibres are identical in terms of all types of staining).

The dorsal peripheral white fibres were typified by large, variable diameters. In contrast, the second population of white fibres were of a regular, rounded shape and small size, and they were present in very small numbers. These characteristics not usually observed in white fibres; hence the separation of the two groups into separate subpopulations.

The fourth fibre type were small diameter fibres, situated next to the fin rays adjacent to the smaller white fibres. These fibres corresponded with the myotomal tonic fibres in that they were mATPase stable (Figure 14b), although not as stable as the white or pink oxidative fibres. They differed from their myotomal counterparts in that they stained negatively for oxidative enzyme activity (Figure 6), glycogen (Figure 10b) and lipid (Figure 14d). The tonic fibres seen in the dorsal musculature were also characterised by a small size and regular shape, in comparision to the tonic myotomal fibres which were variable

in terms of both factors.

In all fibre types within the dorsal fin musculature, a persistent pattern of mATPase staining was observed for pre-incubation durations of five (Figure 12) to twenty minutes (Figure 14b); and at both pH levels of 10.4 (Figures 12, 13a), and 10.5 (Figures 13b, 13c, 14a, 14b).

Fibre types present in the myotome

The myotome of the seahorse is most unusual. The dermal scales are unique among teleost fishes, in that they are modified into a series of bony plates. Caudal, anal, ventral and pelvic fins are not present, and the myotome itself is radically reduced to a narrow prehensile form which is ineffective in locomotion. Within this myotome, further cartilage structures bisect the musculature at a horizontal alignment consistent with the lateral line, and also through the vertical axis. This arrangement effectively divides the myotome into four separate quarters, each with its own arrangement of muscle fibres.

The different fibre types in the myotome of the seahorse were divided into fairly distinct zones when highlighted for mATPase activity.

The bulk of the myotome was composed of large diameter fibres identified as fast (white) on the basis of their size, position in the myotome and staining characteristics. These fibres showed high stability at alkaline pH (ATPase) at pH levels of both 10.4 (Figure 13a) and 10.5 (Figure 13b). They displayed very little oxidative enzyme activity (Figure 8a, 8b, 9a), and stained weakly for glycogen (Figure 9b) and lipid (refer Figure 14d: dorsal white fibres stain identically to those in the myotome).

The second fibre type was positioned peripherally, adjacent to the lateral midline of the fish in a triangular wedge. In addition, peripheral dorsal (Figure 13b) and ventral (Figure 13a, 13c, 14a) wedges were observed, oriented at the level of the vertical axis. They showed a moderately positive result for oxidative enzyme activity (Figure 9a), glycogen (Figure 8b) and lipid, and displayed stable myofibrillar ATPase activity (Figure 13a, 13b, 13c, 14a).

The fact that these fibres were positioned peripherally at the level of the

lateral line in the myotome, and displayed positive staining for oxidative enzyme activity, lipid and glycogen suggested that they might have been slow red fibres. However, subsequent ultrastructural examination revealed virtually no mitochondria in these peripheral cells, therefore discounting this possibility. Further evidence to the contrary was the large range of variation in the diameter of these fibres- red fibres usually constitute a population of uniform dimensions. On the basis of these observations, they were defined as a type of tonic, or small diameter fibre.

In the distal region of the myotome (tail tip), the same pattern of fibre distribution was observed. However, the ventral wedge of small diameter fibres was much more pronounced (Figure 7, 13c).

The position of each fibre type in the myotome was constant.

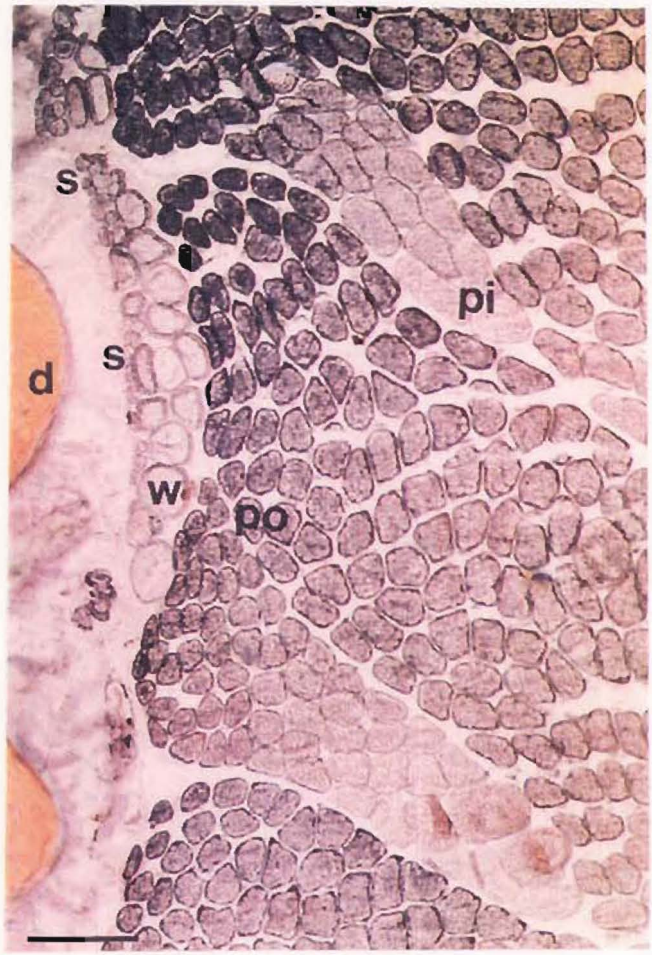


Figure 6. Dorsal fin muscle stained for oxidative enzyme activity.

po = pink oxidative fibres, pi = pink intermediate fibres,
w = white fibres, s = small diameter fibres. d = dorsal fin ray.
Scale bar = 0.2mm.

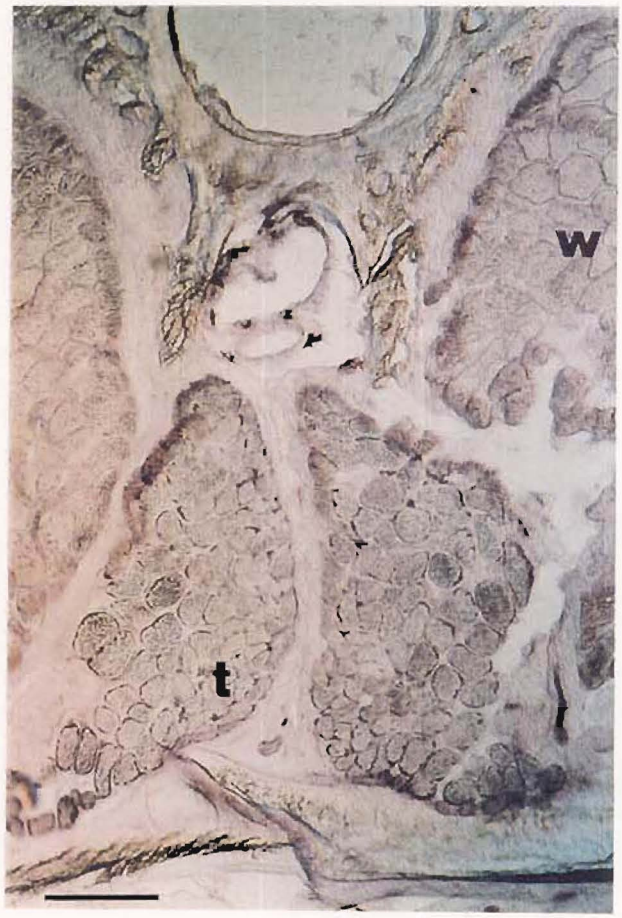


Figure 7. Distal myotomal muscle stained for oxidative enzyme activity.

t = tonic fibres, w = white fibres, d = dorsal fin ray.
Scale bar = 0.2 mm.

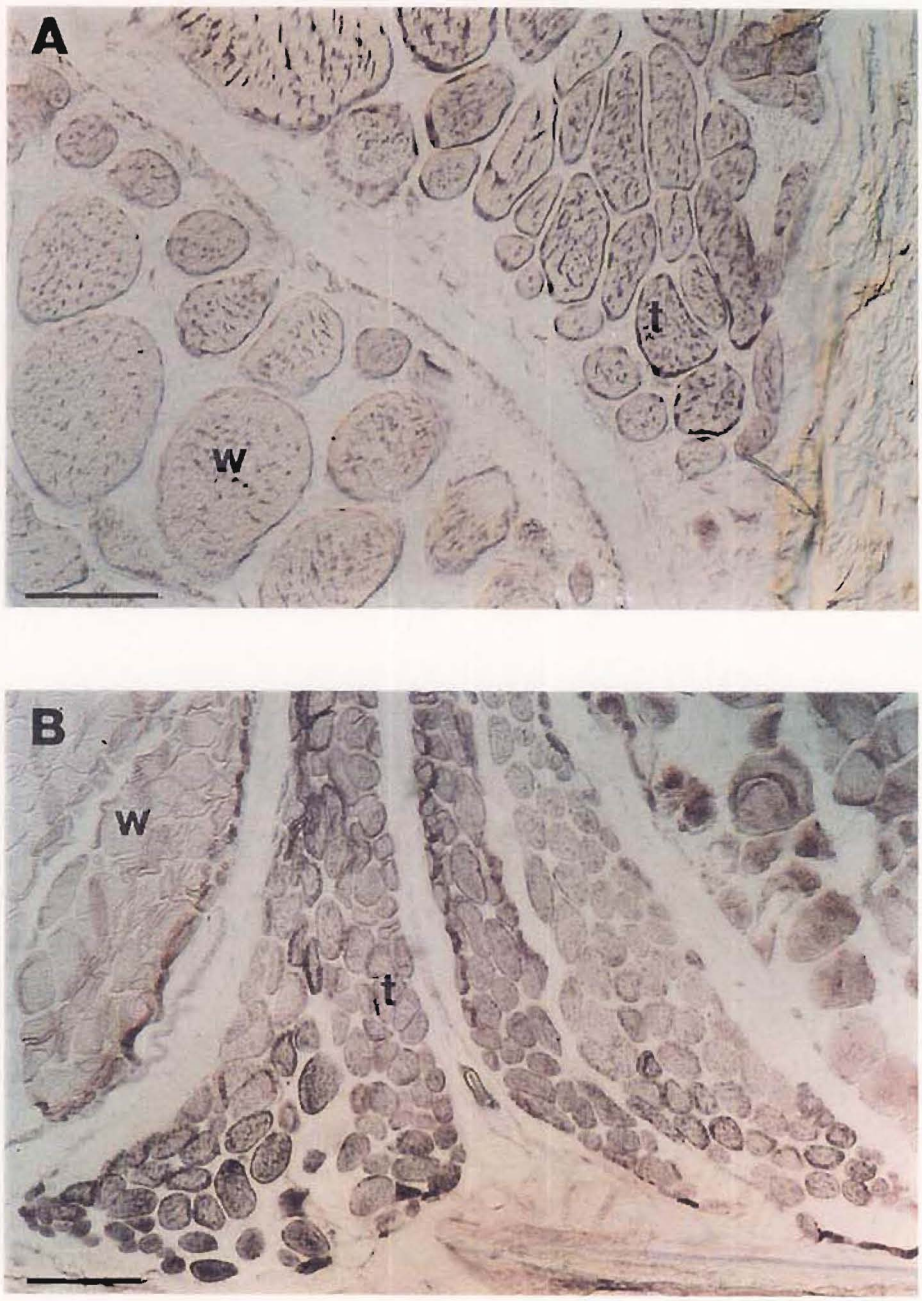


Figure 8. Mid myotomal muscle stained for oxidative enzyme activity.

A. Lateral wedge of tonic fibres. t = tonic fibres, w = white fibres. Scale bar = 0.05 mm.

B. Ventral wedge of tonic fibres. t = tonic, w = white. Scale bar = 0.2 mm.

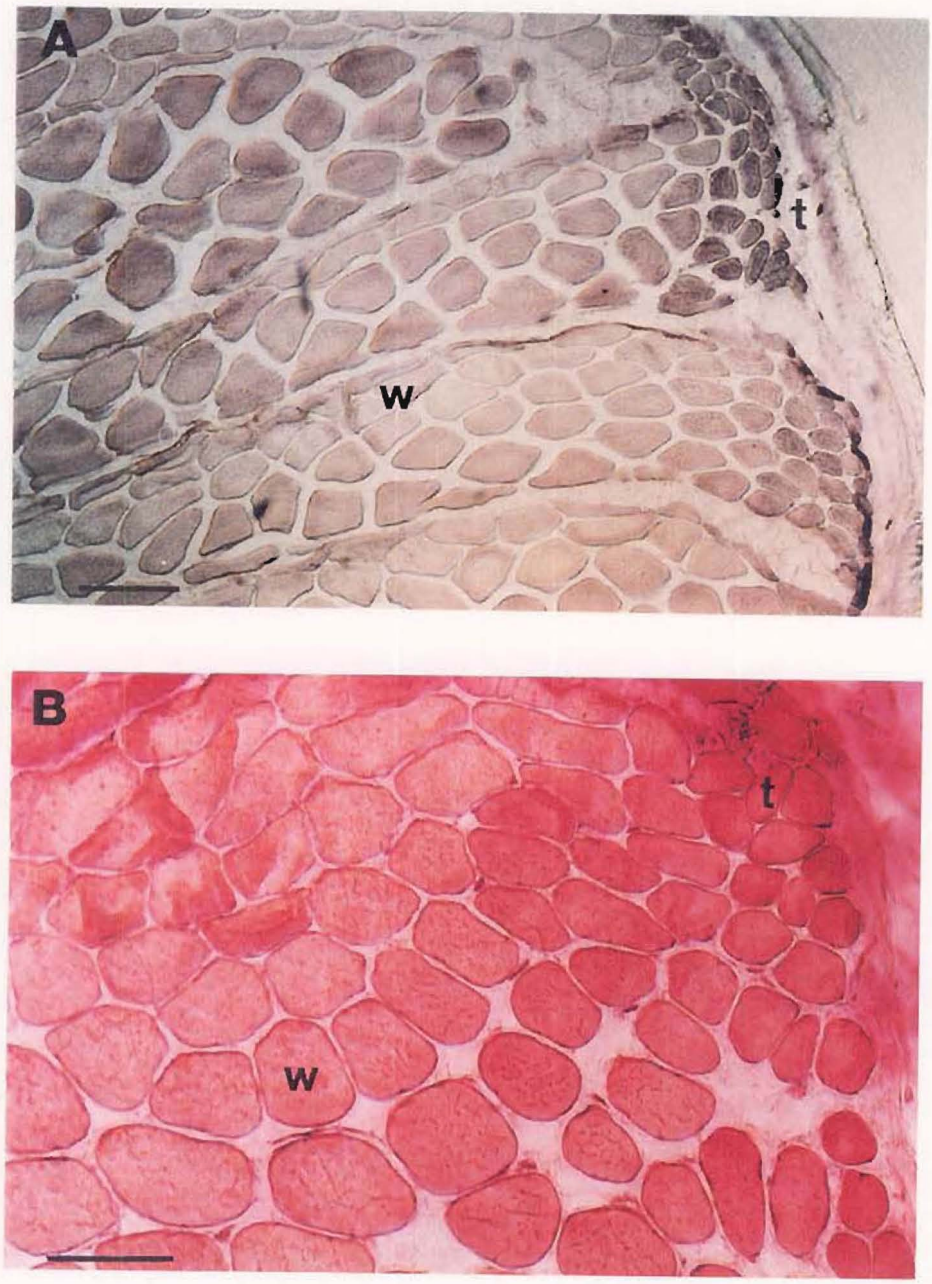


Figure 9A. Mid myotomal muscle stained for oxidative enzyme activity.

t = tonic fibres, w = white fibres.
Scale bar = 0.2 mm.

Figure 9B. Mid myotomal muscle stained for glycogen.

t = tonic fibres, w = white fibres.
Scale bar = 0.1 mm.

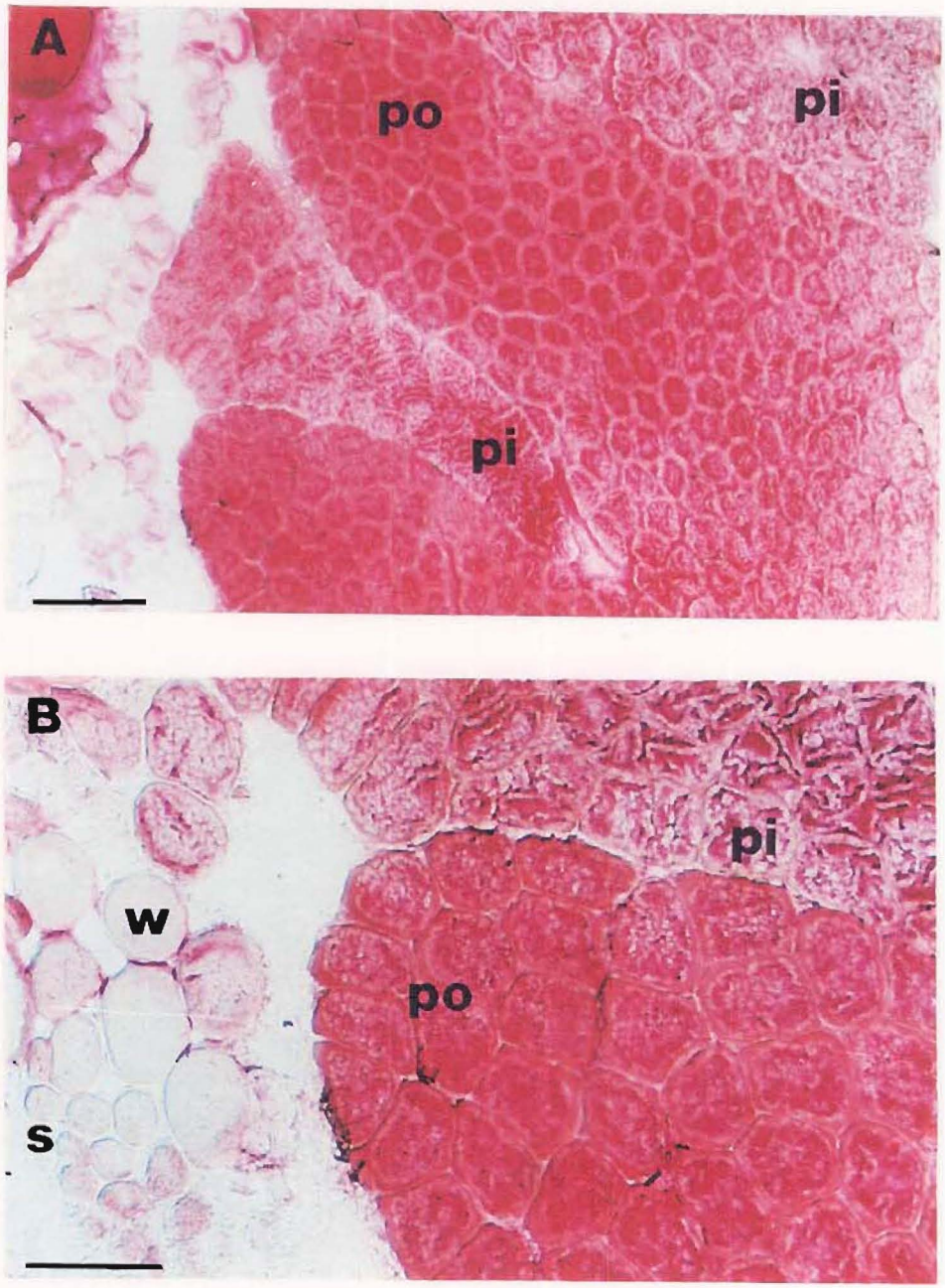


Figure 10. Dorsal fin muscle stained for glycogen.

A. po = pink oxidative fibres, pi = pink intermediate.
Scale bar = 0.2 mm.

B. po = pink oxidative fibres, pi = pink intermediate,
w = white, s = small diameter.
Scale bar = 0.1 mm.

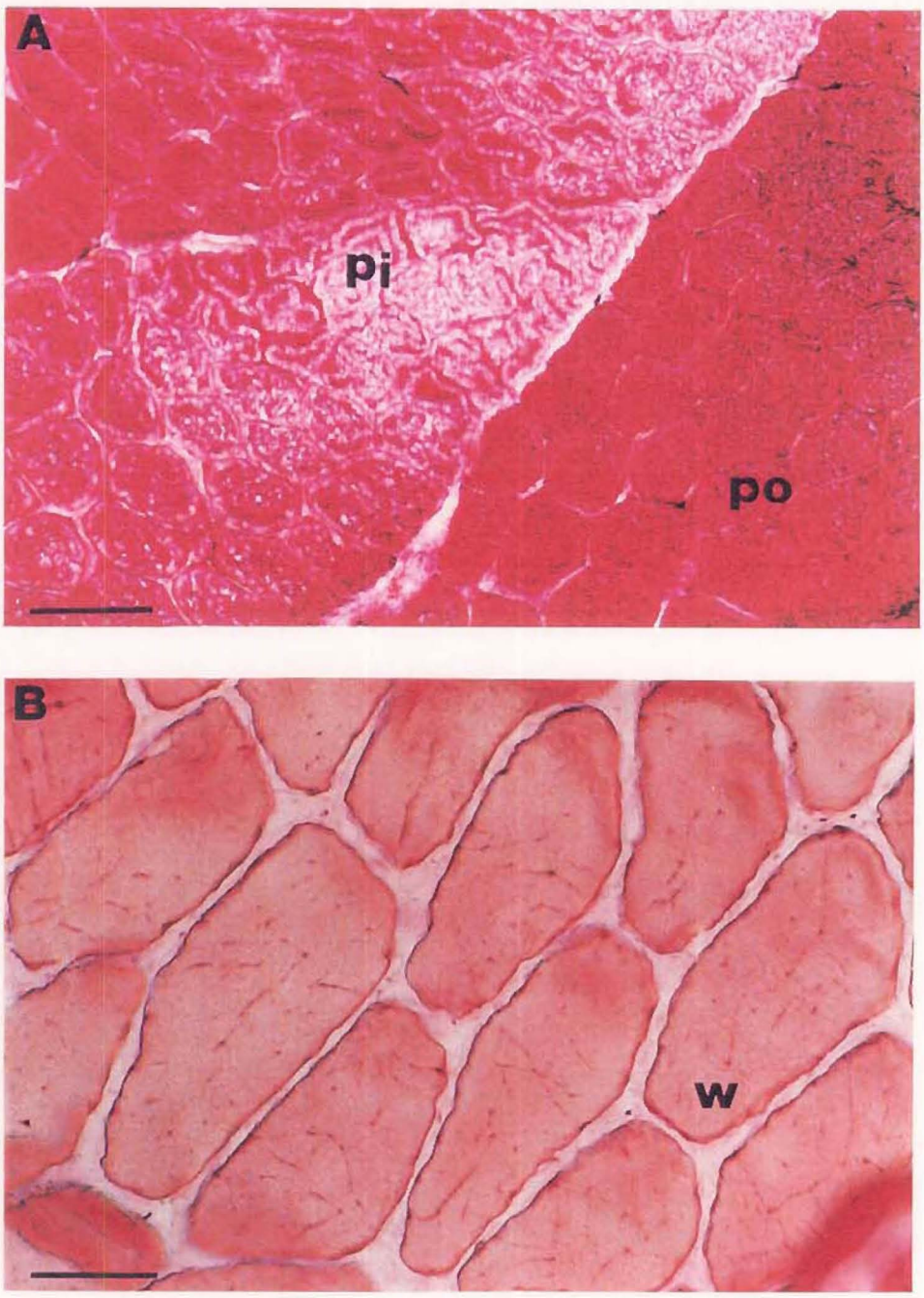


Figure 11. Dorsal fin muscle stained for glycogen.

A. po = pink oxidative fibres, pi = pink intermediate.
Scale bar = 0.05.

B. w = white fibres.
Scale bar = 0.05.

Figure 12. Dorsal fin muscle stained for mATPase activity.

(pH = 10.4, pre-incubation time = 5 minutes).

po = pink oxidative fibres, pi = pink intermediate,
w = white.

Scale bar = 0.2 mm.

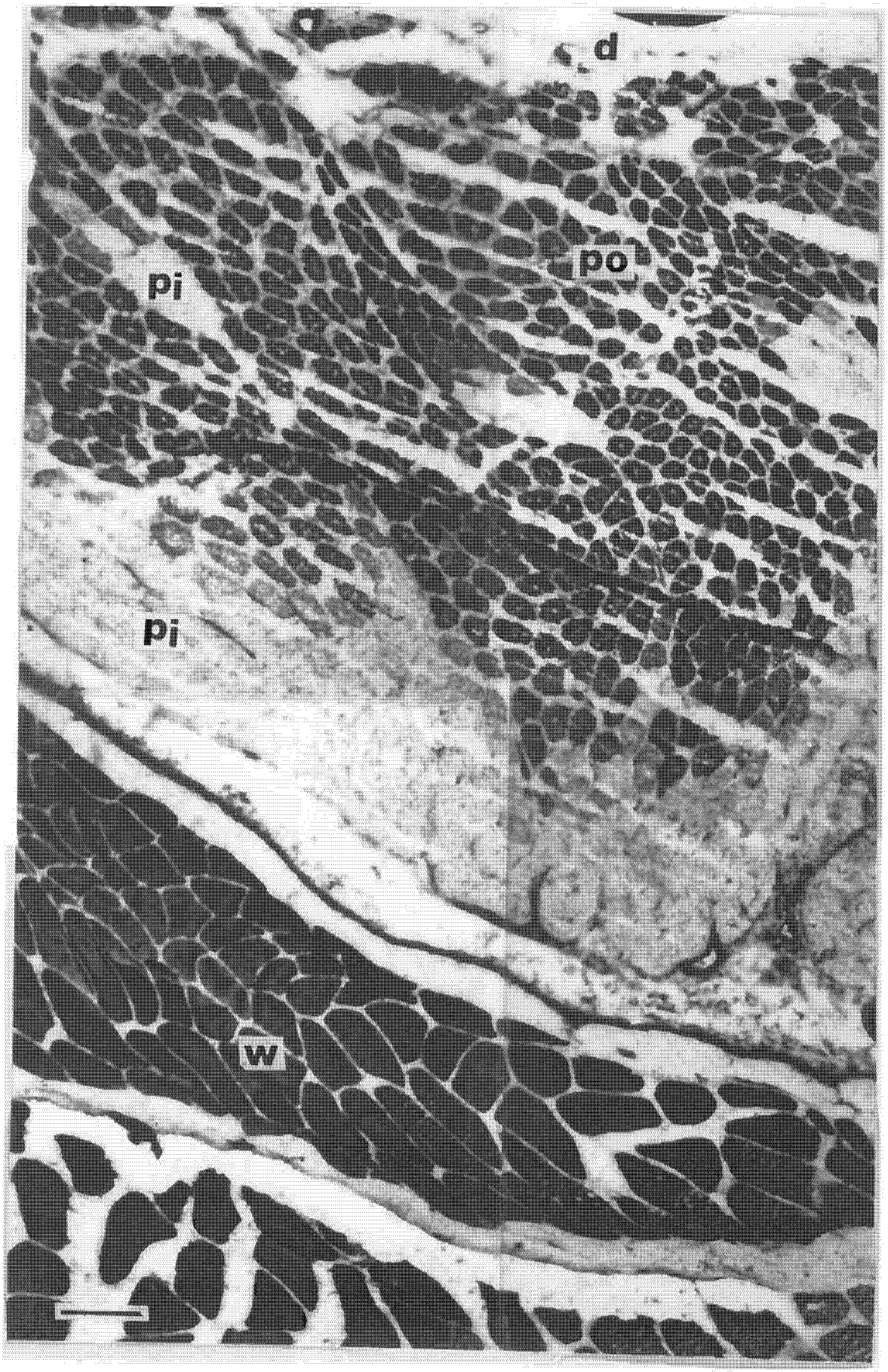

d
po
pi
pi
w

Figure 13A. Mid myotomal muscle stained for mATPase activity.

(pH = 10.4, pre-incubation time = 10 minutes).

t = tonic fibres, w = white fibres.
Scale bar = 0.05.

Figure 13B. Distal myotomal muscle stained for mATPase activity.

(pH = 10.5, pre-incubation time = 10 minutes.

t = tonic fibres, w = white fibres.
Scale bar = 0.1 mm.

Figure 13C. Distal myotomal muscle stained for mATPase activity.

(pH = 10.5, pre-incubation time = 15 minutes).

t = tonic fibres, w = white fibres.
Scale bar = 0.2 mm.

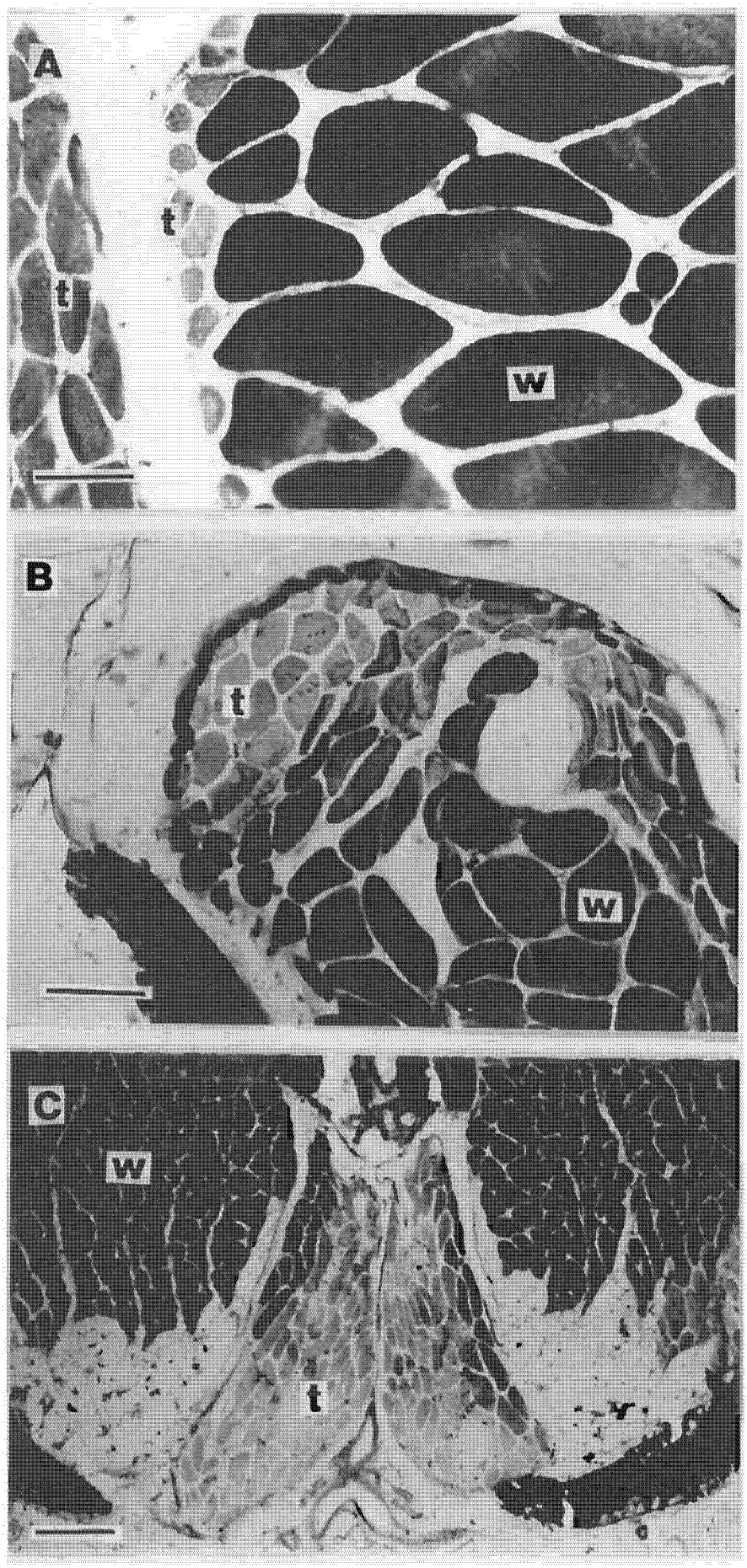

A
t
t
w
B
t
w
C
w
t

Figure 14A. Mid myotomal muscle stained for mATPase activity.

(pH = 10.5, pre-incubation time = 20 minutes).

t = tonic fibres, w = white fibres.
Scale bar = 0.2 mm.

Figure 14B. Dorsal fin muscle stained for mATPase activity.

(pH = 10.5, pre-incubation time = 20 minutes.

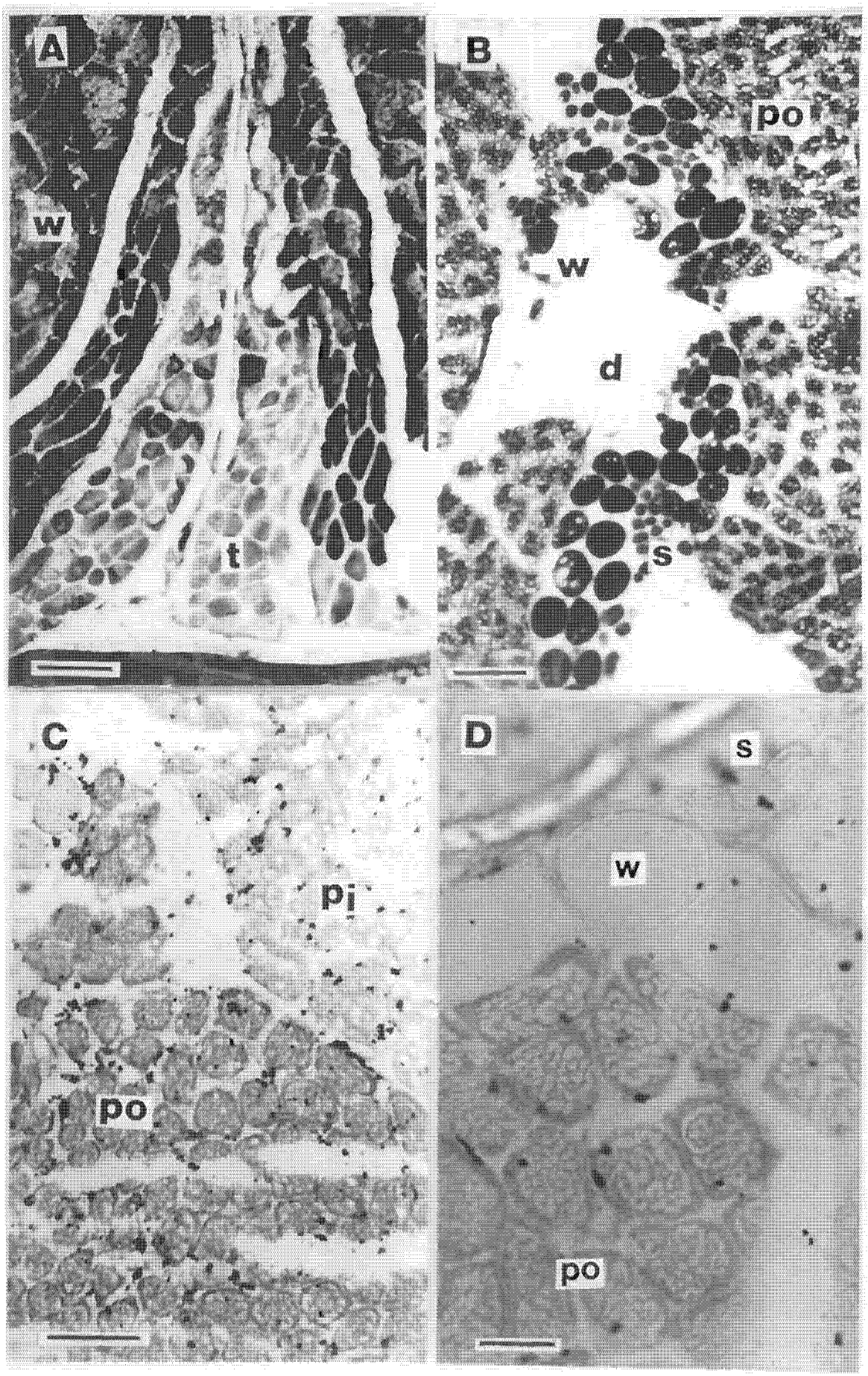
po = pink oxidative fibres, w = white,
s = small diameter, d = dorsal fin ray.
Scale bar = 0.2 mm.

Figure 14C. Dorsal fin muscle stained for lipid.

po = pink oxidative fibres, pi = pink intermediate.
Scale bar = 0.1 mm.

Figure 14D. Dorsal fin muscle stained for lipid.

po = pink oxidative fibres, w = white, s = small diameter.
Scale bar = 0.02 mm.


A
w
t
B
po
w
d
s
C
pi
po
D
s
w
po

Ultrastructure

Ultrastructure of the muscle cells

White fibres

The electron microscope proved to be an invaluable asset in the identification and description of the different muscle fibre types.

White fibres in the dorsal fin and the myotome were identical. The myofibrils were arranged in a closely packed, regular pattern (Figures 15a, 15b, 15c); enclosed by a voluminous sarcoplasmic mass bound by the sarcolemma (Figures 15b, 15d). The spaces in the myofibrils were filled with sarcoplasmic reticulum, which was extensive and well-developed (Figures 15a, 15c, 15e). The fibres were multinucleated, the nuclei being usually peripheral. Caveolae were observed all along the inner peripheral sarcolemma (Figure 15f).

The white fibres differed in the extent of glycogen distributed between the myofibrils. Some cells contained abundant visible glycogen granules, while others did not (Figure 15b). No lipid droplets were seen in the white fibres.

Mitochondria were not present in substantial numbers. They were typically limited to the periphery of the cells (Figures 15b, 15d, 15f). At the longitudinal orientation, mitochondria were positioned within the myofibrils at the level of the A-I junction. At the terminal point of the myofibril ribbon, they were specifically oriented between adjacent z-lines at the level of the A-I junction (Figure 15d). The z-lines and m-lines of the sarcomere were well-defined (Figures 15d, 15e).

The T-system was not observed to be particularly extensive; the triads being fairly irregularly distributed. Triads were located at the level of the A-I junction

(Figure 15e).

Collagen was abundant in the extracellular space (Figure 15d). Nerves were not often observed.

Pink oxidative fibres

The pink oxidative fibres were radically different from the white fibres. The myofibrils were tightly packed in discrete, well-defined bundles, which were separated by large expanses of cytoplasm, and surrounded by mitochondria (Figure 16a). Large distances between the myofibril bundles and the sarcolemma were also observed (Figures 16d, 16e).

The fibres were multinucleated, the nuclei being either central (Figure 16b), or peripheral (Figure 17d). Nuclei were always surrounded by abundant mitochondria.

Glycogen granules were evident throughout the cytoplasm (Figure 17f).

The mitochondria in these fibres were not only observed to surround the myofibrils, but to be present in large numbers at the periphery of the cell (Figures 16c, 16d, 16e). Mitochondria were variable in size, albeit predominantly large; and well-developed, containing clearly-delineated cristae (Figures 17a, 17e, 17f). At the longitudinal orientation, mitochondria were also seen to be present within the myofibrils (Figures 17c, 17f); while those surrounding the myofibrils were observed to be aligned at the level of the z-line (Figure 17f). (At the transverse orientation, mitochondria were observed to completely surround the myofibrils).

The T-system was more extensive than that of the white fibres. Triads were located at the level of the z-line (Figure 17f). The z-lines and m-lines were present (Figure 17c, 17f).

Caveole were present at the cell periphery (Figure 17e). Large vesicles were also observed within the cytoplasm; again often near the sarcolemma (Figure 17e).

The oxidative cells were well vascularised, containing numerous capillaries (Figures 16c, 16d). Nerves and nerve endings were also often observed (Figure 16f). Collagen fibrils and fibroblasts were also abundant (Figure 16f).

Pink intermediate fibres

The ultrastructure of the intermediate pink fibres was identical to that of the oxidative pink fibres. However, the two populations differed in that the intermediate fibres contained significantly fewer mitochondria (Figure 18a), and blood vessels.

The myofibrils were arranged in the same pattern as in the oxidative cells (Figure 18a). The T-system was also more extensive in these fibres compared to the white (Figure 18b, 18e); similarly, the mitochondria were aligned at the level of the z-band when observed at the longitudinal orientation (Figure 18b, 18e). Triads were also located at the z-line (Figure 18d).

The mitochondria were observed to be as well-developed in the intermediate fibres as in the former population (Figure 18b). There were very few mitochondria located at the periphery of the cell (Figure 18e).

All other features of these cells were identical to the oxidative pink fibres.

Dorsal tonic fibres

Tonic fibres in the dorsal fin were ultrastructurally different in comparision to those in the myotome. Dorsal fin tonic fibres were always small; hence they have been referred to as 'small diameter fibres' (an analogous title to 'tonic').

The myofibrils of these cells were enclosed in a large area of cytoplasm extending to the cell periphery (Figure 17b). The myofibrils were tightly packed, and the sarcoplasmic reticulum appeared to be well developed. Mitochondria were rarely observed and almost exclusively peripheral (Figure 17b).

The T-system of these fibres was not well developed. Triads were situated at the A-I junction.

Myotomal tonic fibres

In contrast to the dorsal fin tonic fibres, the myotomal tonic fibres were of a variable size (Figures 19a, 19b, 20a). The area of cytoplasm between the myofibrils and the sarcolemma was considerably smaller, and the areas of sarcoplasmic reticulum between the myofibrils were not as large. In these respects, as in many others, the tonic fibres in the myotome were similar to the white fibres. Tonic fibres were distinguished from white fibres on the basis of size, and by the fact that the individual characteristic appearances of the transverse sections were different.

The myofibrils in these fibres were tightly packed, with a reasonably well-developed sarcoplasmic reticulum (Figure 19a). The mitochondria were located both peripherally and centrally (Figure 19b). Nuclei were also located

peripherally (Figure 19b), and centrally. Collagen was extensively distributed (Figure 19a, 20b); and fibroblasts were often observed (Figures 19a, 20b). Nerves were commonly seen. A large nerve tract was observed among the fibres (Figure 19a).

At the longitudinal orientation, the tonic fibres of the myotome were again similar to the white fibres. The T-system was not as extensive as that of the pink fibres. Triads were located at the A-I junction (Figures 20d). Mitochondria were located within the myofibrils, and oriented at the A-I junction (Figures 20d, 20f). Mitochondria were not as highly developed as in the pink fibres.

The z-lines and m-lines were both well-defined, although the z-line was jagged (Figures 20d, 20f).

Single tonic fibres of a different type were also observed (Figures 20c, 20e). They were not considered to be of interest in this study as they were present in such insignificant numbers.

Figure 15.

A. Transverse section (TS) of a white muscle fibre. Note regularly packed myofibrils (mf) and well-developed sarcoplasmic reticulum (sr) throughout.
Scale bar = 1 μm.

B. TS of two white fibres. The cell on the right contains more glycogen, identified as black grains between the myofibrils. Note peripheral mitochondria (→).
Scale bar = 1 μm.

C. TS of a white fibre, demonstrating the liberally branched sarcoplasmic reticulum (→), and regularly packed myofibrils.
Scale bar = 400 nm.

D. Longitudinal section (LS) of terminal portion of a white fibre myofibril. Note mitochondria (m) located between the z-lines. The "wave" pattern of the cell edge is probably an artifact of the preparation.
Scale bar = 500 nm.

E. TS of a triad (t), located at the a-i junction (ai), the characteristic location for triads in white fibres. Also of note; the z-line (z), and sarcoplasmic reticulum (s).
Scale bar = 200 nm.

F. TS of mitochondria (m) adjacent to the a-i junction, next to the z-line (z) Note caveolae (→).
Scale bar = 200 nm.

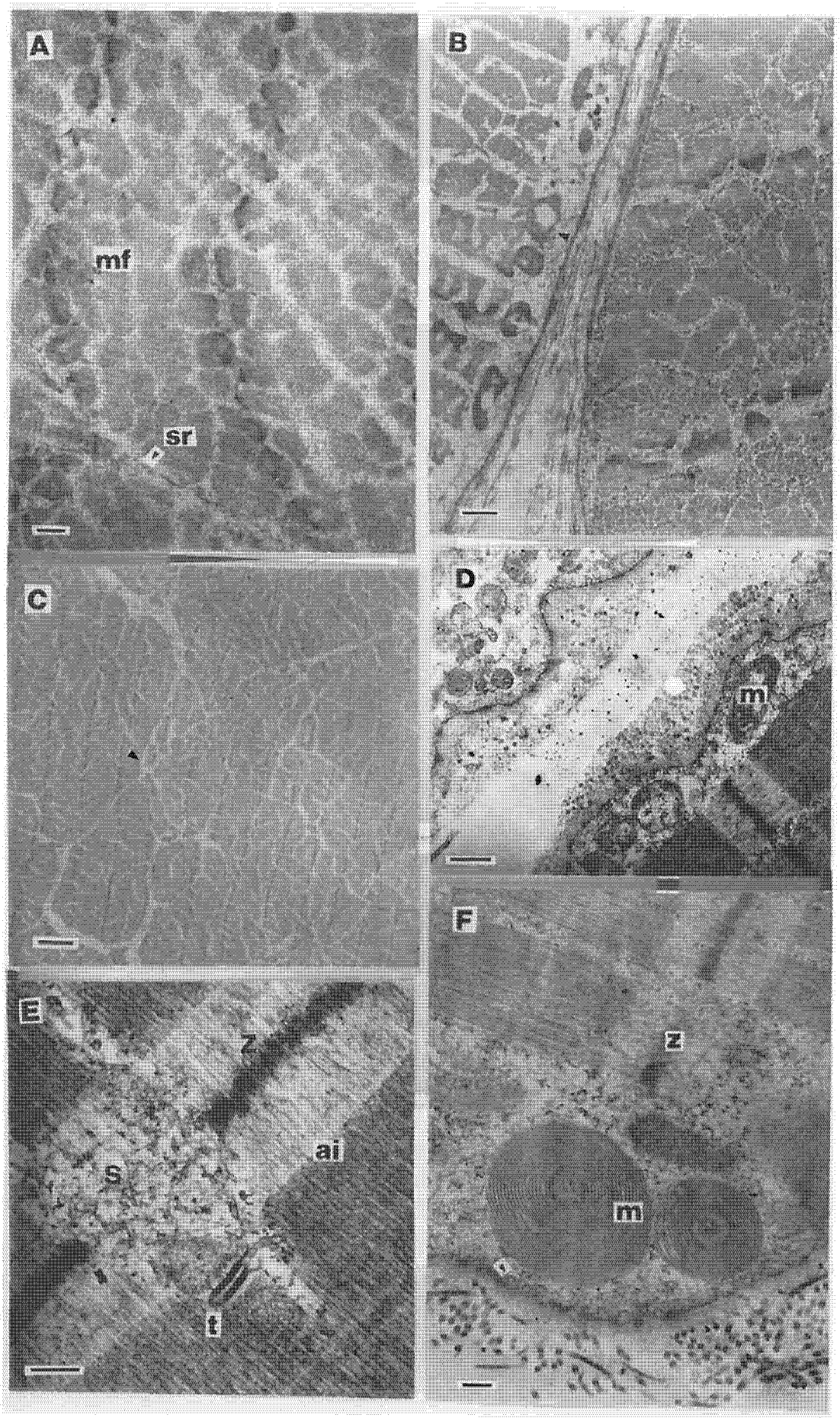

A
mf
sr
B
C
D
m
E
Z
ai
s
t
F
z
m

Figure 16.

A. Transverse section (TS) of part of a pink oxidative muscle fibre. Note the numerous mitochondria (→) surrounding the myofibrils (m), and dispersed throughout the cytoplasm (c).
Scale bar = 2μm.

B. TS of pink oxidative fibre. In the middle of the picture is a central nucleus surrounded by mitochondria.
Scale bar = 2μm.

C. TS of periphery of a pink oxidative cell (rather contracted). Note well developed peripheral mitochondria (m), collagen fibrils (→), and part of a capillary (c).
Scale bar = 500 nm.

D. TS of a capillary near the periphery of an oxidative pink fibre. Note large numbers of peripheral mitochondria near the blood vessel.
Scale bar = 2μm.

E. Clusters of peripheral mitochondria. Note the large expanse of cytoplasm between the myofibrils and the cell edge (TS).
Scale bar = 4μm.

F. TS showing nerves (n) near the edge of an oxidative pink fibre. Also of note; a nerve ending (ne), and a fibroblast (f), located near the nerve.
Scale bar = 400 nm.

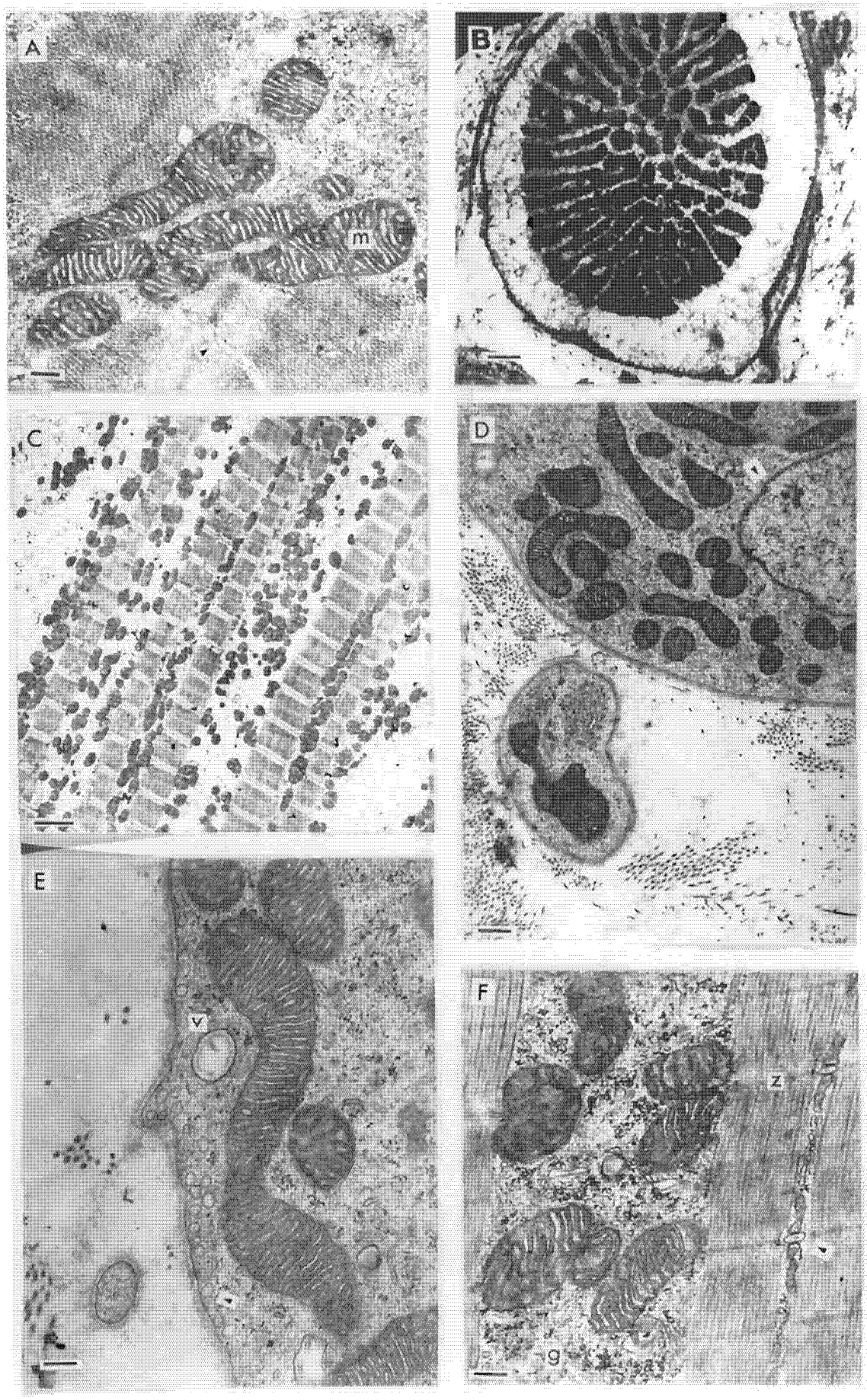

A
m
B
C
D
E
v
F
z
g

Figure 17.

A. TS of pink oxidative fibre. Note well developed mitochondria (m), located in close proximity to the sarcoplasmic reticulum (→), between muscle bundles.
Scale bar = 200 nm.

B. TS of a small diameter fibre from the dorsal fin.
Scale bar = 200 nm.

C. Longitudinal section through a pink oxidative fibre. Note that mitochondria not only surround the myofibrils, but are also present within them. They are oriented at the level of the z-lines.
Scale bar = 2μm.

D. TS of a pink oxidative fibre, cell edge. Note peripheral nucleus (→). The small membrane-bound "cell" containing mitochondria, is probably a cross-section of the very terminal end of another oxidative cell.
Scale bar = 500 nm.

E. TS of pink oxidative cell periphery, demonstrating a large elongated mitochondrion. Note well-developed cristae. v = a large vesicle. Peripheral caveolae (→) are found along the entire distance of the cell edge.
Scale bar = 500 nm.

F. LS of a pink oxidative fibre. Mitochondria and triads (t) are oriented at the level of the z-line (z). Note the presence of glycogen (g).
Scale bar = 200 nm.

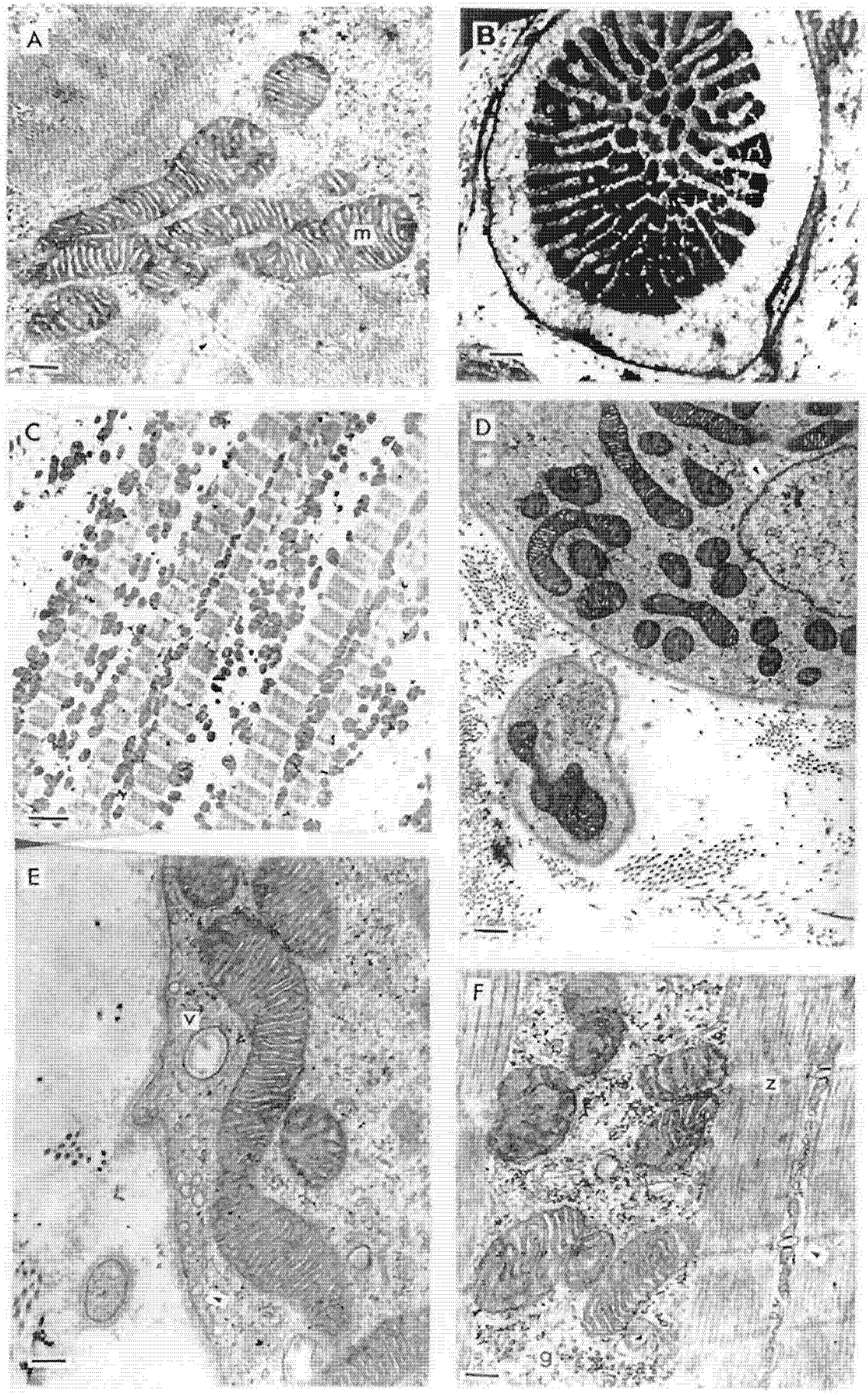

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Figure 18.

A. TS of a dorsal pink intermediate fibre. Note the identical myofibril arrangement, but lack of mitochondria in these fibres; in comparision to the oxidative fibres.
Scale bar = 400 nm.

B. LS of intermediate pink fibre. Note well devoloped mitochondria (m), and triads (→), at the level of the z-line (z).
Scale bar = 1 μm.

C. TS of pink intermediate fibre. Note large expanse of cytoplasm (c), myofibrils (m), and sarcoplasmic reticulum (s), and mitochondria (→).
Scale bar = 1 μm.

D. LS of intermediate pink fibre, showing triads (t) at the z-line.
Scale bar = 200 nm.

E. LS of intermediate pink fibre. Triads and mitochondria are oriented at the z-line. Note the large expanses of sarcoplasmic reticulum near the periphery of the cell.
Scale bar = 1 μm.

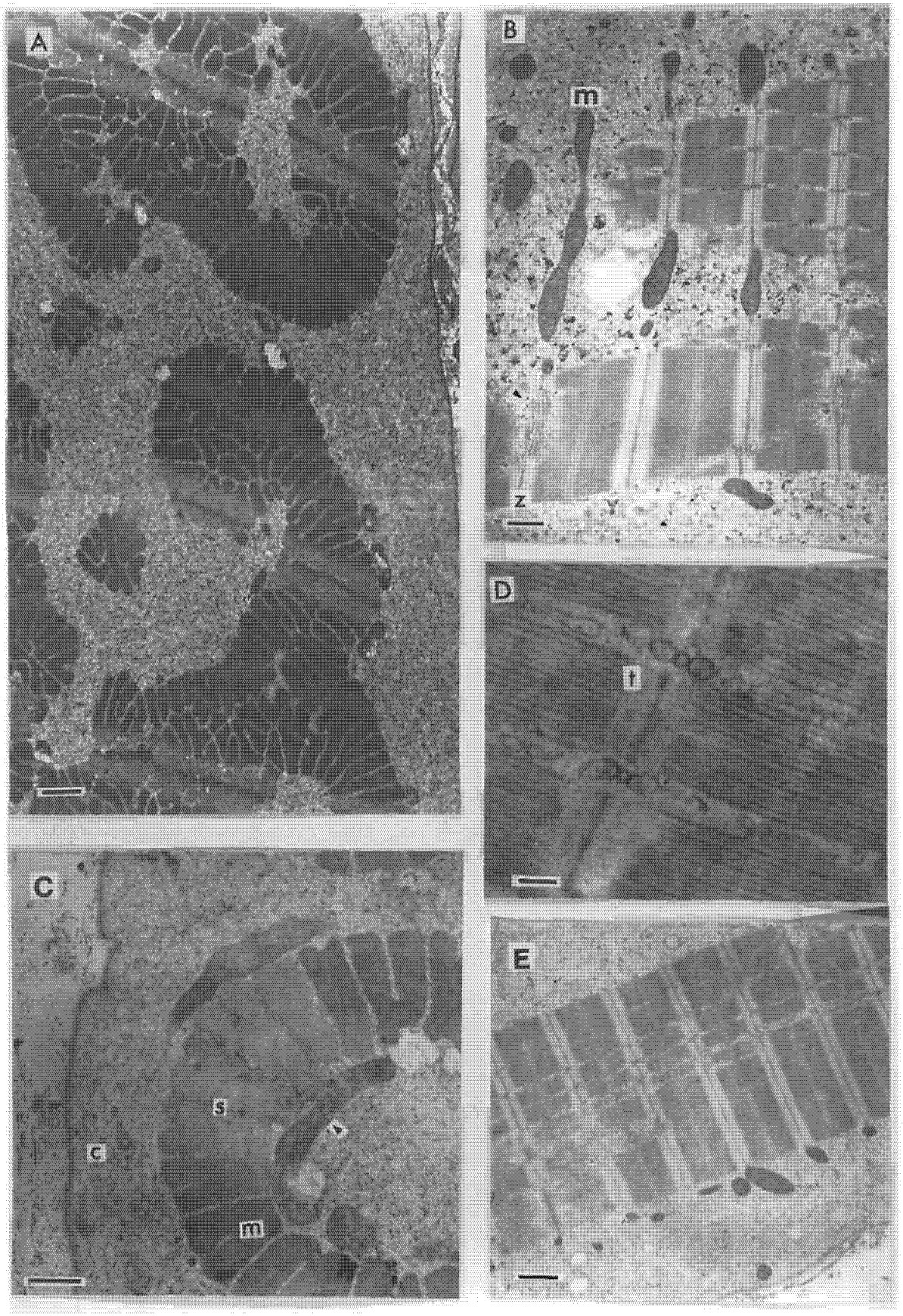
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Figure 19.

A. TS of a tonic fibre from the myotome. –> = sarcoplasmic reticulum. Also of note: fibroblasts (f), considerable amounts of collagen fibrils, and a nerve tract (nt).
Scale bar = 400 nm.

B. TS of a larger tonic fibre in the same region of the myotome as Figure 24a. m = mitochondria (also note the presence of peripheral mitochondria). –> = peripheral nucleus.
Scale bar = 400 nm.

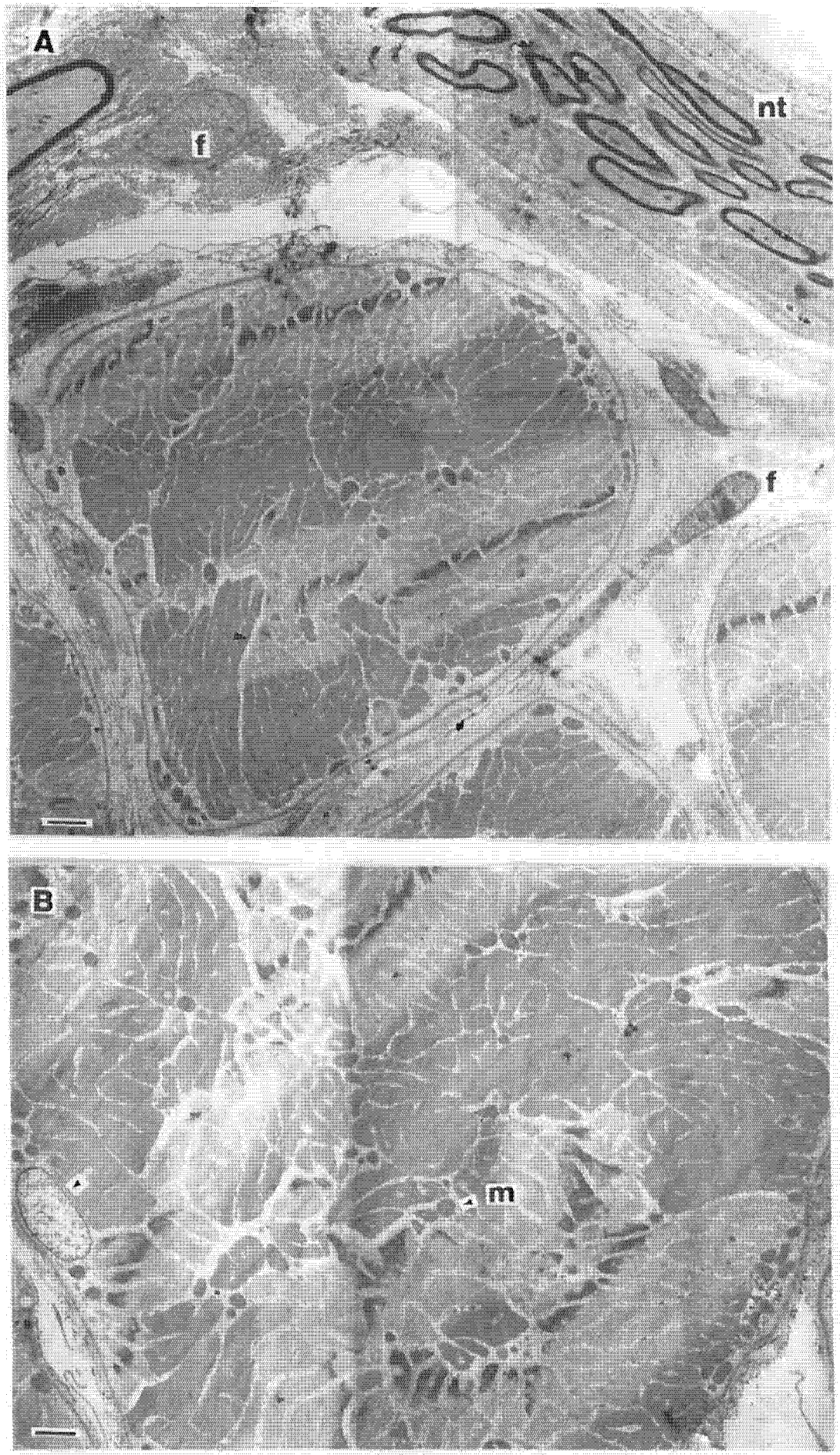

A
f
nt
f
B
m

Figure 20.

A. TS of a small tonic fibre.
Scale bar = 400 nm.

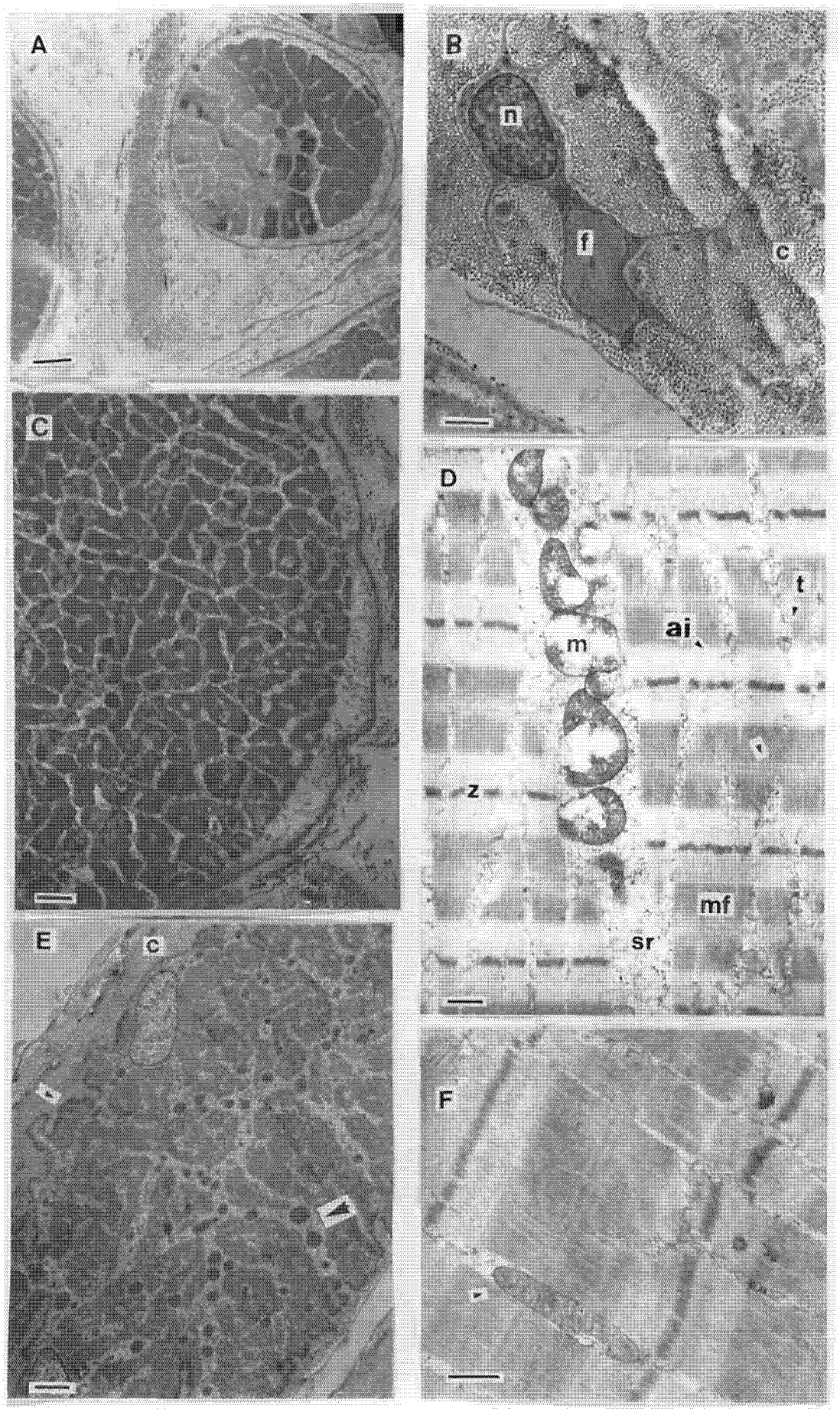
B. TS section of a fibroblast (f) situated near the periphery of a tonic fibre. Note the large nucleus (n). The vast fields of collagen (c) (a product of the fibroblasts), are typical of tonic muscle.
Scale bar = 500 nm.

C. TS of a tonic fibre of a different type. These fibres were very rarely seen. On the basis of this fact, they were not thought to play a significant role in the musculature. Alternatively, the appearance may be an artifact of the preparation.
Scale bar = 1 μm.

D. LS of tonic fibre. Note mitochondria (m) located within the sarcoplasmic reticulum (sr) between the myofibrils (mf). Triads (t) are located at the level of the a-i junction (ai). Also of note: jagged z-line (z), and well-defined m-line (→).
Scale bar = 500 nm.

E. TS of another unusual tonic fibre type, containing wide channels of sarcoplasmic reticulum and large mitochondria (large →). Also note the contracted cell membrane (small →), and the surrounding field of collagen fibrils (c).
Scale bar = 1 μm.

F. TS of a large, elongated mitochondrion (→) positioned at the a-i band of a tonic fibre.
Scale bar = 500 nm.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Ultrastructure of extracellular components

Vascularisation

The levels of vascularisation in the seahorse muscle fibres were differentiated according to fibre type.

In accordance with their relatively scarce mitochondria, lipids, and oxidative enzymes, white fibres displayed very little vascularisation. The pink intermediate and tonic fibres also showed very limited vascularisation. Pink oxidative fibres were the only group in which blood vessels were present to a significant extent.

In the oxidative pink muscle, arterioles were rarely seen; however capillaries were numerous and ranged in size. They were always seen in close association with the muscle cells, often in almost direct contact. In many cases, they were situated in a groove in the sarcolemma (Figures 21a, 21b). Capillaries were also commonly observed in small spaces between more than one individual muscle cell (Figure 21c).

Pericytes were often observed, located between the capillaries and the sarcolemma (Figure 21b).

The capillaries were always associated with collagen and fibroblasts (Figures 21a, 21b, 21c).

The capillaries were composed of a single layer of endothelial cells joined by desmosomes (Figure 21f). Their walls contained no muscular or fibrous elements. Pinocytotic vesicles were present in considerable densities throughout the endothelial cells, forming extensive channels within the cytoplasm. They were distributed to the periphery of the cells (Figure 21f). In addition, caveolae were observed to be present; located specifically at the level of the cell membrane

(Figure 21f).

Arterioles were occasionally observed (Figure 21e). The endothelium of these vessels was thick compared to that of the capillaries, and was strengthened by bundles of tonofilaments. The endothelial lining consisted of cuboidal cells surrounded by a substantial basement membrane. These endothelial cells formed a complete ring around the lumen of the arteriole, and were held together by desmosomes at the approximate midpoints of the cell walls. The number of demosomes varied according to the thickness of the point of contact betwen the endothelial cells; in thin areas of contact, one desmosome was usually seen, but at contact points of a thicker surface area, two to four desmosomes could be seen at regularly spaced intervals. Mitochondrial density in the arterioles was sparse, and their structures were not well developed, showing litle evidence of cristae.

A layer of smooth muscle was seen to surround the epithelium, separated by a basement membrane. Outpockets from the endothelial cells functioned to anchor the two layers. The smooth muscle was characterised by sparse contractile filaments on the intimal side of the cell (Figure 21e).

Venules were also more rarely observed than capillaries (Figure 21d). They were usually far larger than either the capillaries or the arterioles, and were composed of a single layer of squamous epithelium, instead of thick cuboidal cells and a smooth muscle layer.

Figure 21.

A. Vascularisation of the musculature. TS of a capillary located in a groove of the cell periphery. Note that myofibrils are not always located this close to the sarcolemma.
Scale bar = 1 μm.

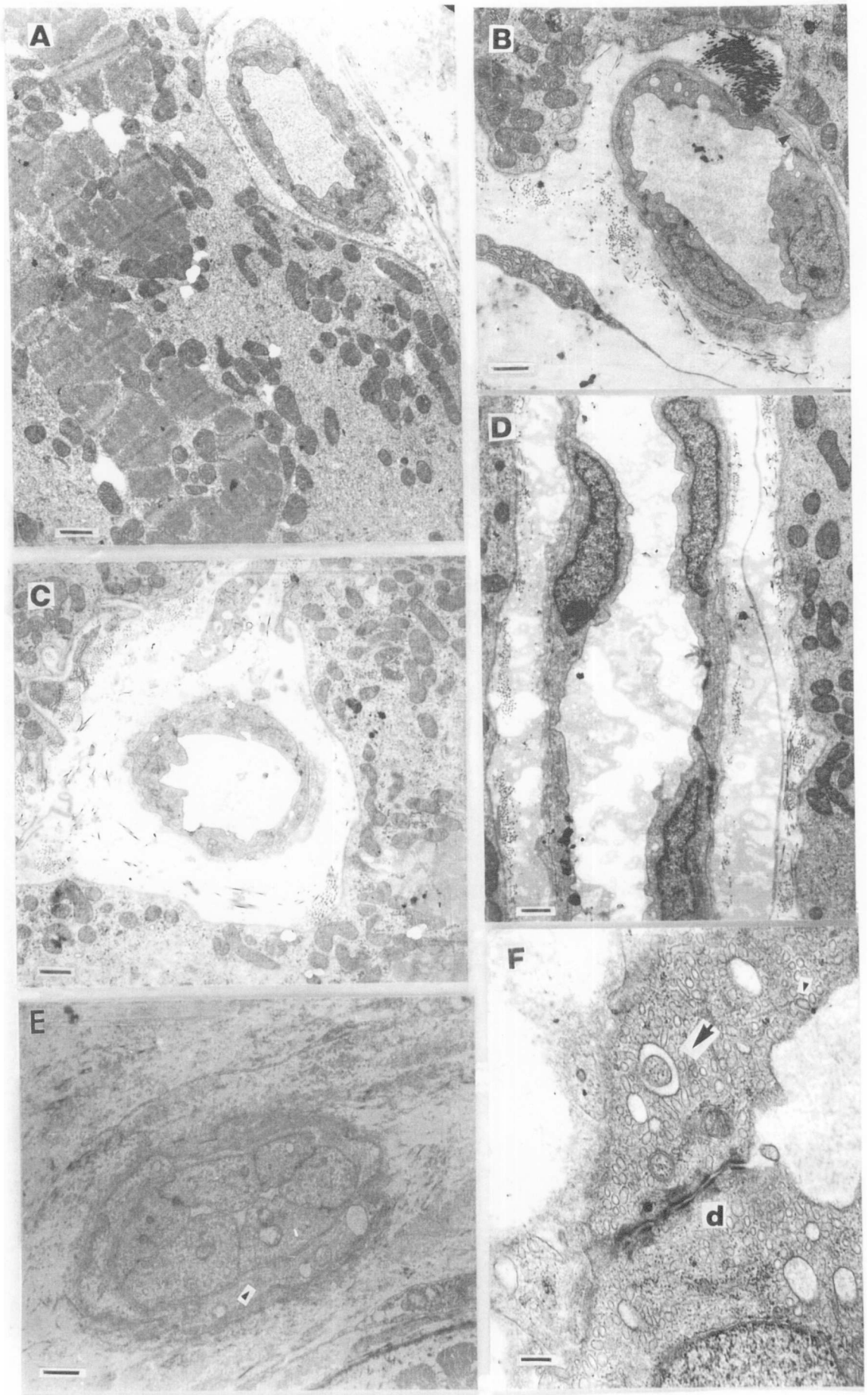
B. Another (larger) capillary, also located in a groove of the cell endothelium. Note the profuse collagen connective tissue (and adjacent fibroblast); and the pericyte located at the point of contact between cell and vessel (→).
Scale bar = 1 μm.

C. TS of a capillary positioned in a small space between two oxidative muscle fibres.
Scale bar= 1 μm.

D. TS of part of a large venule, located between two oxidative fibres.
Scale bar = 1 μm.

E. TS of an arteriole, found in an area of white muscle fibres. Surrounding the vessel is a layer of smooth muscle (→).
Scale bar = 1 μm.

F. TS of part of a capillary, showing the numerous vesicles (large arrow), and peripheral caveolae (small arrow). Also note four desmosomes (spot welds) joining the adjacent cuboidal cells of the arteriole (d).
Scale bar = 200 nm.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Innervation

Nerves were frequently observed in the extracellular space, especially in that of the oxidative pink fibres. They were identified by their 'racetrack' appearance. Some were myelinated (Figures 22a, 22b); while others were not (Figure 22a).

Numerous nerve endings were observed throughout the extracellular space, always in close contact with the muscle cells (Figure 22c); and sometimes fitting into smooth depressions in the muscle fibre (Figure 22d). No infoldings in the sarcolemna were observed. Nerves and nerve endings were often surrounded by collagen fibrils.

Extracellular Connective Tissue

One of the most striking features of the extracellular substance was the extent of the connective tissue and the profusion and development of the associated fibroblasts. The collagen fibrils were present in large fields surrounding the muscle fibres (Figure 20b), the blood vessels (Figure 21e), and the nerves (Figure 16f). In the only nerve tract observed, the spaces between the nerves were filled with collagen (Figure 22a).

Associated with the tremendous amount of connective tissue in the extracellular space were numerous, widely distributed fibroblasts, the cells most commonly found in connective tissue (Figures 22e, 22f, 22g).

The fibroblasts were characterised by abundant, irregularly branched cytoplasm containing lots of endoplasmic reticulum, a well developed golgi complex, and a large, ovoid nucleus with a prominent nucleolus and fine chromatin (Figure 20b). They were variable in structure and size (Figures 22e, 22f, 22g).

Figure 22.

A. TS of a nerve tract within the tonic musculature. Note myelinated nerves (m), unmyelinated nerves (u), and collagen (c) interspersed between the nerves. The entire nerve tract is surrounded by a membrane and lots of collagen connective tissue.
Scale bar = 1 μm.

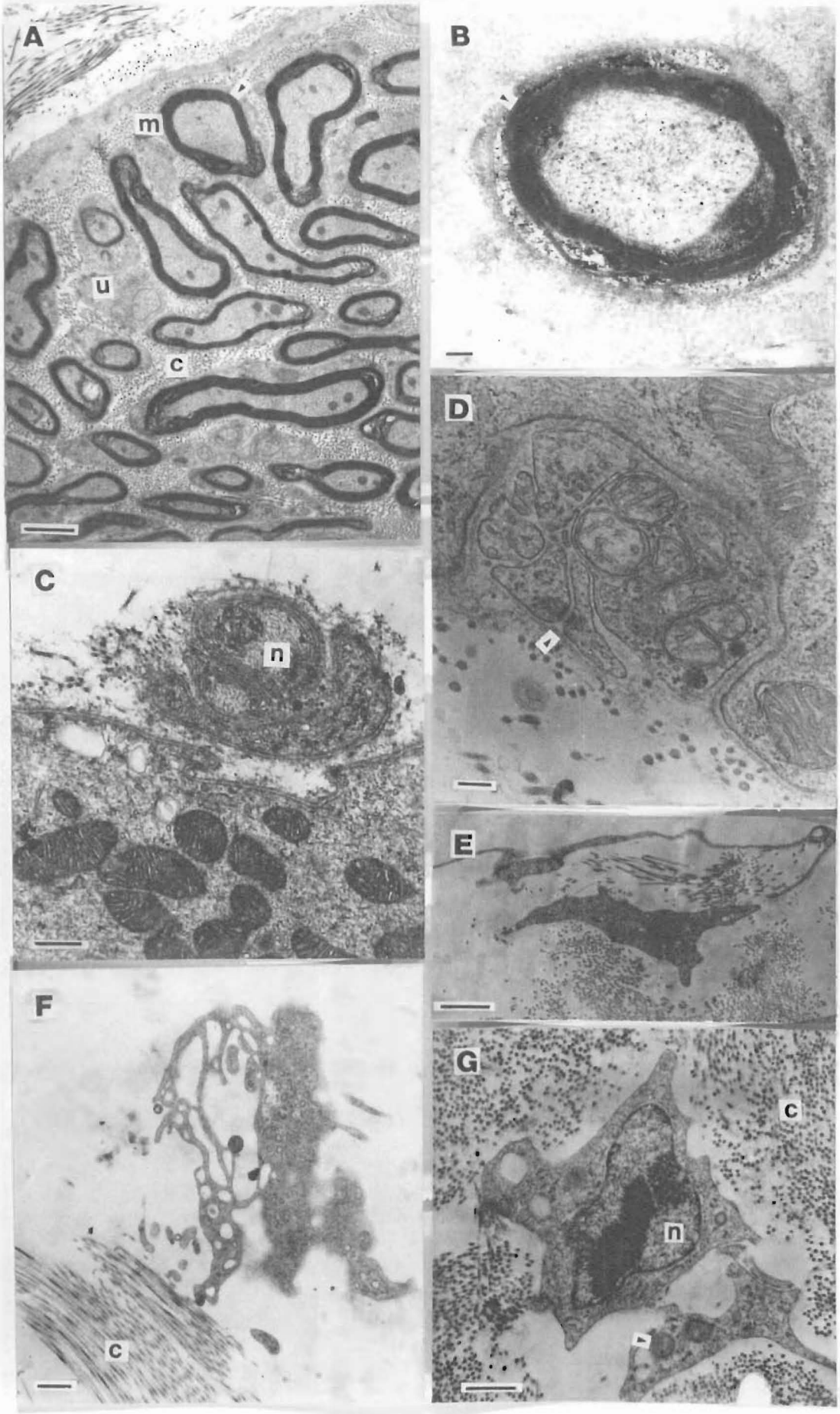
B. TS of a myelinated nerve. Nerves are identified by their 'racetrack' patterned myelin sheath.
Scale bar = 200 nm.

C. A nerve ending, positioned close to the cell periphery (TS). Note the nucleus (n), mitochondria (→), and attendant collagen (c).
Scale bar = 500 nm.

D. TS of a nerve ending situated in an endothelial depression in the cell edge. Note the desmosome (→).
Scale bar = 200 nm.

E. TS of another fibroblast.
Scale bar = 1 μm.

F. TS of a fibroblast. c = collagen fibrils.
Scale bar = 500 nm.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Proportions of fibre types

The proportions of different fibre types from the three regions of investigation (see Figure 5) are shown in Table 1 and Figure 23. Table 2 provides a source of comparision between different fibre types of other fish species.

The proportion of oxidative musculature in the dorsal fin was noted to increase significantly as the fish increased in size ($p<0.05$). There was no oxidative musculature in the myotome whatsoever.

Proportions of white fibres in the dorsal fin were recorded to be decreasing with increasing fish length ($p<0.05$). However, no significant variations in the proportions of the small diameter fibres, the 'small white' fibre subpopulation, or the intermediate pink fibres of the dorsal fin were observed to be occurring as the fish increased in size.

In the mid tail region, white fibre proportions were observed to be increasing at a significant level between size classes (21.2-22.5 cms) and (23.5 to 24.5 cms) only ($p<0.05$). This trend was reflected by a significant decrease in myotomal tonic fibre proportions between these two size classes ($p<0.05$). Between size classes (23.5-24.5 cms) and (28.5-29.5 cms), no significant differences were observed in the fibre proportions of either tonic or white fibres.

The trends observed in the distal tail region parallel the results from the mid tail region. Proportions of white fibres were significantly lower ($p<0.05$) in the dorsal fin that either the mid or the distal tail regions. Proportions of white fibres in the latter two myotomal regions were not significantly different from each other. Proportions of tonic fibres in the mid tail region were also not significantly different from those in the distal tail region.

Table 1. Proportions of different muscle fibre types of *Hippocampus abdominalis*.

Fish size class (cm)		21.5-22.5	23.5-24.5	28.5-29.5	Pooled data
Dorsal pink oxidative	avg	39	41	45	
	st.d	13	9	11	
Dorsal pink intermediate	avg	7	8	7	
	st.d	2	2	8	
Dorsal total oxidative	avg				49.0
	st.d				3
Dorsal white (myotomal)	avg	48	44	42	
	st.d	12	7	14	
Dorsal white (near rays)	avg	4.5	5.5	4.5	
	st.d	2	1.2	3	
Total dorsal glycolytic	avg				49.3
	st.d				3
Dorsal small diameter	avg	1.5	1.5	1.5	1
	st.d	0.5	0.5	2	0.5
Mid tail tonic	avg	33	25	27	28.3
	st.d	1.2	2	4.5	3.8
Mid tail white	avg	67	75	73	72
	st.d	3	2	7	4.2
Distal tail tonic	avg	35	28	29	30.7
	st.d	2	2	6	3.7
Distal tail white	avg	65	72	71	69.3
	st.d	1	2	5	3.8

Table 2. Proportions of myotomal red muscle (per cent total muscle) for several fish species compared to the myotomal red muscle proportions of *Hippocampus abdominalis*.

(All references except for *Hippocampus* from Greer Walker and Pull, 1975).

Family	Species	Mean % red muscle
Syngnathidae	*Hippocampus abdominalis*	0
Scombridae	*Scomber colias* (Gmelin)	29.8
	Scomber scombrus (Linnaeus)	18.8
Clupeidae	*Engraulis encrasicolus* (Linnaeus)	17.2
	Alosa follax (Lacepede)	21.5
	Sardina pilchardus (Walbaum)	28.9
Carangidae	*Trachurus trachurus* (Linnaeus)	18.3
Sparidae	*Pagellus bogaraveo* (Brunnich)	15.7
Cyclopteridae	*Cyclopterus lumpus* (Linnaeus)	14.8
Mugilidae	*Crenimugil labrosus* (Risso)	14.5
Squaloidae	*Squalus acanthias* (Linnaeus)	14.3
Ammodytidae	*Ammodytes marinus* (Raitt)	12.9
Cheilodipteridae	*Epigonis telescopus* (Risso)	12.7
Gadidae	*Gadus poutassou* (Risso)	12.6
	Gadus merlangus (Linnaeus)	11.7
	Gadus luscus (Linnaeus)	12.1
	Gadus minitus (Linnaeus)	11.5
	Gadus pollachius (Linnaeus)	3.3
	Gadus virens (Linnaeus)	10.8
	Gadus morhua (Linnaeus)	17.0
	Gadus aeglefinus (Linnaeus)	12.5
	Brosme brosme (Ascanius)	1.9
	Phycis blennoides (Brunnich)	24.9
	Merluccius merluccius (Linnaeus)	5.5
	Molva molva (Linnaeus)	1.6
	Gaidropasarus vulgaris (Cloquet)	9.7
	Ciliata mustela (Linnaeus)	13.0

Table 2. (continued).

Family	Species	mean % muscle
Syngnathidae	*Hippocampus abdominalis* (red)	0
Argentinidae	*Argentina silus* (Ascanius)	11.3
	Argentina sphyraena (Linnaeus)	7.5
Zeidae	*Zeus faber* (Linnaeus)	9.3
Pleuronectidae	*Limanda limanda* (Linnaeus)	10.3
	Platichthys flesus (Linnaeus)	12.0
	Pluronectes platessa (Linnaeus)	11.4
	Microstomus kitt (Walbaum)	9.8
	Glyptocephalus cynoglossus (Linnaeus)	9.9
	Hippoglossoides platessoides (Fabricius)	7.0
	Hippoglossus hippoglossus (Linnaeus)	1.6
Morinae	*Mora moro* (Risso)	10.0
	Lepidon eques (Gunther)	7.5
Anguillidae	*Anguilla anguilla* (Linnaeus)	8.8
Soleidae	*Solea solea* (Linnaeus)	10.9
	Buglossidium luteum (Risso)	5.4
Osmeridae	*Osmerus eperlanus* (Linnaeus)	6.2
	Mallotus villosus (Muller)	8.5
Bothidae	*Scophthalmus maximus* (Linnaeus)	11.1
	Lepidorhombus whiffiagonis (Walbaum)	3.3
Lophiidae	*Lophius piscatorius* (Linnaeus)	7.2
Triglidae	*Eutrigla gumardus* (Linnaeus)	6.3
	Aspitrigla cuculus (Linnaeus)	7.6
Macrohamphosidae	*Macrohamphosus scolopax* (Linnaeus)	6.4

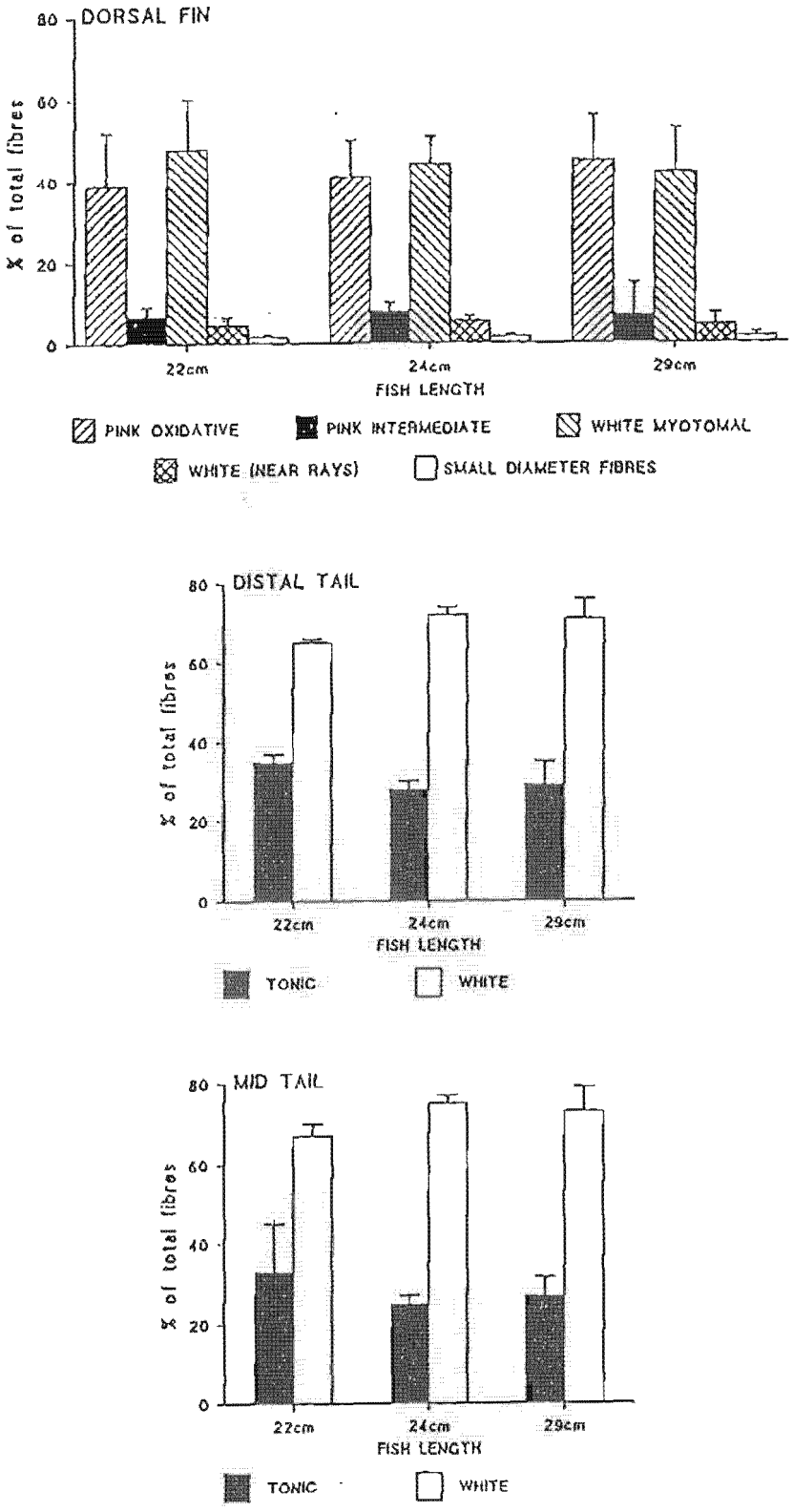


Figure 23. Proportions of the different fibre types in *Hippocampus abdominalis*.

Percentages of cell components

The results of this section of the study are presented in Table 3 and Figure 24. Table 4 provides a source of comparison to other species of fish.

Percentages of mitochondria were found to be significantly higher in the dorsal pink oxidative muscle fibres than in any other fibre type ($p<0.05$). Mitochondrial densities were not significantly different between the other fibre types in the dorsal fin.

Mitochondrial densities were not significantly different in any of the myotomal white or tonic fibres, except for the distal tail white fibres which were significantly different from all the other groups in the myotome ($p<0.05$).

The percentage of cytoplasm in the mid tail white fibres was not significantly different from that of the white fibres in either the distal tail or the dorsal fin. Neither was it significantly different from that of the tonic fibres in either region of the tail. However, percentages of cytoplasm in white and tonic fibres of both myotomal regions were significantly different from those of both subpopulations of pink fibres ($p<0.05$). In addition, the percentage of oxidative pink cytoplasm was significantly lower than that of the intermediate pink fibres ($p<0.05$).

In the dorsal fin, the proportions of myofibrils were highest in the intermediate pink fibres ($p<0.05$). No significant differences were observed between the myofibril proportions in the white fibres of the myotome, in either the mid tail region, or that of the distal tail. Myotomal white myofibril densities were also not significantly different from those in the dorsal fin musculature.

Distal tail tonic fibre myofibril densities were not significantly different from the values obtained from the mid tail region.

Table 3. Percent proportions of cell components in the different fibre types of *Hippocampus abdominalis*.

Cell Component		Muscle	Cytoplasm	Mitochondria
Dorsal pink oxidative	avg	31.8	36	32.2
	st.d	9.4	10	5.7
Dorsal pink intermediate	avg	54.7	43.5	2.4
	st.d	6.2	6.7	1.2
Dorsal white (myotome)	avg	67	22	1
	st.d	8.6	6.3	0.9
Mid tail white	avg	73.0	25.9	2.0
	st.d	6.8	6.9	0.9
Mid tail tonic	avg	71.3	26.6	2.1
	st.d	6.2	6.8	0.8
Distal tail white	avg	72.6	27.1	0.3
	st.d	7.1	6.8	0.4
Distal tail tonic	avg	70.4	27.2	2.0
	st.d	6.7	6.4	1.2

Table 4. Mitochondrial volumes (%) in different fibre types of several fish species compared to *Hippocampus abdominalis*.

Fibre type	Red	Pink	White	Tonic	Reference
Acipenser stellatus	30	3.7	0.7	-	1
Chimaera spp.	52 ± 2.1	1.8 ± 0.7	0.3 ± 0.5	-	2
Etomoptrus spp.	30.4 ± 4.8	7.2 ± 5.3	0.5 ± 0.6	-	2
Galeus spp.	34.1 ± 4.9	16.3 ± 5.3	0.9 ± 1.1	-	2
Mugil cephalus	31.43 ± 1.17	-	4.23 ± 1.13	-	3
N. barbatulus	25 ± 0.06	-	0.09 ± 0.01	0.03 ± 0.01	4
Perca fluviatilis	21	7	6 -	-	5
Scyliorhinus 1	24.8 ± 3.2	-	0.99 ± 0.17	-	2
Scyliorhinus 2	18.3 ± 6.4	3.9 ±1.8	1.3 ± 0.9	-	2
H. abdominalis (Dorsal)	-		1.0 ± 0.9	*	7
(pink intermediate)		2.4 ± 1.2			7
(pink oxidative)		32.2 ± 5.7			7
		-			7
H. abdominalis (Mid t)	-	-	2.0 ± 0.9	2.1 ± 0.8	7
H. abdominalis (Dis. t)	-	-	0.3 ± 0.4	2.0 ± 0.7	7
Cyprinius carpio	24.8 ± 1.6				6
(large pink)		2.2 ± 0.3	-	-	6
(small pink)		5.9 ± 0.7	-	-	6

* Fibres of this type were not measured as only three were found.

Note: Regions (eg myotome) from which the above values were taken (with the exception of the seahorse) were not listed.

References

1. Kryvi *et al.*, 1980
2. Totland *et al.*, 1981
3. Quaglia, 1980
4. Kilarski *et al.*, 1986.
5. Raamsdonk *et al.*, 1981
6. Akster, 1985
7. (own)

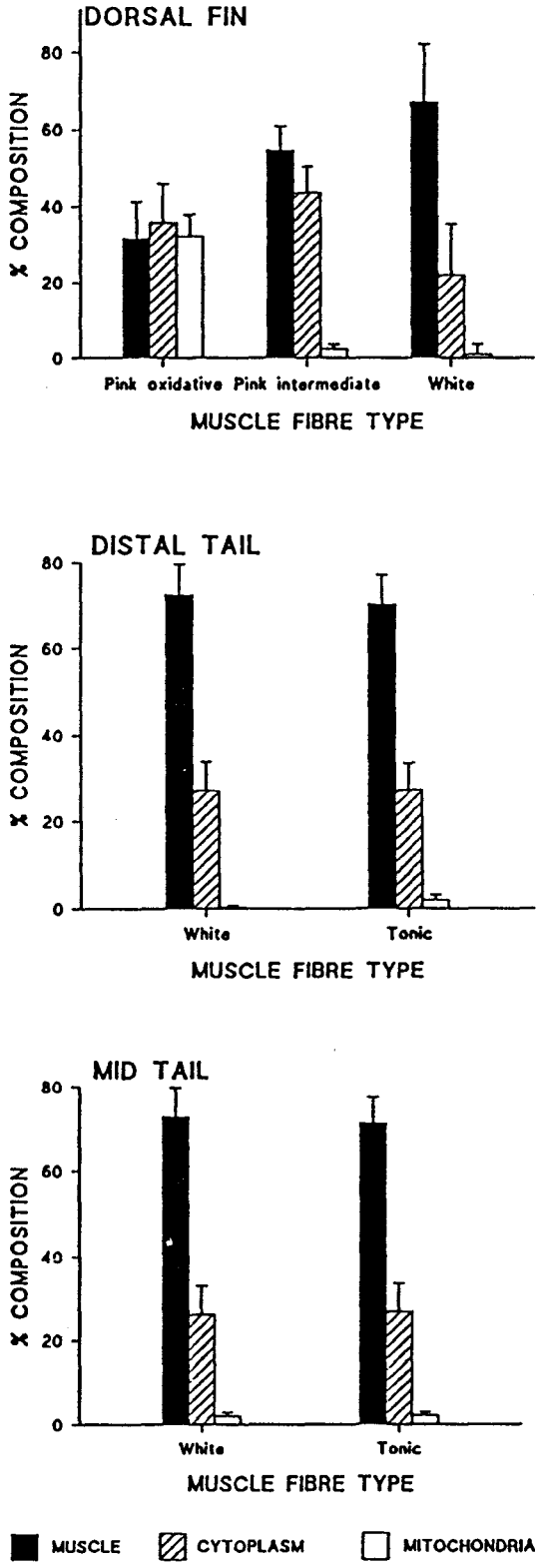


Figure 24. Percentages of cell components in the different fibre types of *Hippocampus abdominalis*.

Fibre Diameters

The size-frequency distributions of the fibre types in the dorsal fin and myotome are shown in Figure 25 and Figures 26a and 26b. Mean diameters of myotomal and dorsal fibres are presented in Table 5 and Figure 27.

In all cases, fibre diameters showed a positive correlation with fish length ($p<0.05$).

In all cases, the diameters of the individual fibre types were significantly different from each other ($p<0.05$).

Small diameter fibres were the smallest fibre type and had a fairly uniform diameter in the dorsal fin, but were far more variable in diameter in the myotome. Small diameter (tonic) fibres were also significantly larger in the myotome than their dorsal counterparts ($p<0.05$).

Dorsal fin white fibres were significantly larger than either the mid tail white fibres ($p<0.05$), or the distal tail white fibres ($p<0.05$).

Pink intermediate fibres were significantly larger than pink oxidative fibres ($p<0.05$).

Table 5. Diameters of the different fibre types in *Hippocampus abdominalis*.

(All measurements in μm).

Fish no.		1	2	3	4	5	6
Length (cm)		**19**	**22**	**24**	**24**	**25**	**29**
Dorsal pink oxidative	avg	56	70	61	64	81	82
	st.d	5	9	14	13	10	17
Dorsal pink intermediate	avg	84	104	104	105	107	130
	st.d	29	14	15	13	13	33
Dorsal white (myotomal)	avg	120	190	223	231	232	242
	st.d	3	7	33	27	35	43
Dorsal white (near rays)	avg	72	81	75	87	90	99
	st.d	9	13	9	19	11	14
Dorsal SDF	avg	25	33	25	32	35	38
	st.d	6	12	10	11	9	9
Mid tail white	avg	82	85	152	156	165	165
	st.d	15	17	22	48	32	30
Mid tail tonic	avg	35	46	71	78	81	86
	st.d	9	15	18	28	23	34
Distal tail white	avg	62	66	84	89	92	101
	st.d	13	10	17	17	17	13
Distal tail tonic	avg	23	27	35	48	60	70
	st.d	9	8	7	17	14	15

Table 6. Mean red and white fibre diameters (μm) in the myotome of several species of fish, compared to the white fibres (size range 19cms to 29cms) in *Hippocampus abdominalis* in the myotome and dorsal fin. Also note comparision between oxidative pink fibres (μm) of *Hippocampus* (range 19cms to 29cms) and red fibres of other species.
(All references except for *Hippocampus* from Greer-Walker and Pull, 1975).

Family	Species	Mean red fibres (μm)	Mean white fibres (μm)
Syngnathidae	*Hippocampus abdominalis* (Dorsal pink oxidative)	56-82 (μm)	
Syngnathidae	*Hippocamus abdominalus* (Dorsal large)	-	120-242
	(Dorsal near rays)	-	72-99
	(Mid myotome)	-	82-165
	(Distal myotome)	-	62-101
Scombridae	*Scomber colias* (Gmelin)	13.1	28.9
	Scomber scombrus (Linnaeus)	22.0	69.0
Clupeidae	*Engraulis encrasicolus* (Linnaeus)	-	45.6
	Alosa follax (Lacepede)	15.9	38.1
	Sardina pilchardus (Walbaum)	24.0	1.0
	Sprattus sprattus (Linnaeus)	22.0	37.0
	Clupea harengus (Linnaeus)	18.0	42
Carangidae	*Trachurus trachurus* (Linnaeus)	23.9	80.9
Sparidae	*Pagellus bogaraveo* (Brunnich)	14.3	47.5
Cyclopteridae	*Cyclopterus lumpus* (Linnaeus)	21.6	50.0
Mugilidae	*Crenimugil labrosus* (Risso)	30.9	9.9
Squaloidae	*Squalus acanthias* (Linnaeus)	36.4	98.8
Ammodytidae	*Ammodytes marinus* (Raitt)	25.6	46.9
Cleilodipteridae	*Epigonis telescopus* (Risso)	13.0	64.9
Gladidae	*Gadus poutassou* (Risso)	32.4	98.4
	Gadus merlangus (Linnaeus)	33.2	80.1
	Gadus luscus (Linnaeus)	35.4	65.8
	Gadus minitus (Linnaeus)	31.3	100.7
	Gadus pollachius (Linnaeus)	24.3	128.0
	Gadus virens (Linnaeus)	51.1	143.8

Table 6. (continued).

Family	Species	Mean red fibres (µm)	Mean white fibres (µm)
Syngnathidae	***Hippocampus abdominalis*** **(Dorsal pink oxidative)**	**56-82**	
Syngnathidae	*Hippocamus abdominalus* (Dorsal large)	-	120-242
	(Dorsal near rays)	-	72-99
	(Mid myotome)	-	82-165
	(Distal myotome)	-	62-101
Pleuronectidae	*Pleuronectes platessa* (Linnaeus)	34.7	158.5
	Hippoglossoides platessoides (Fabricius)	25.8	96.9
	Hippoglossus hippoglossus (Linnaeus)	23.2	73.7
Morinae	*Mora moro* (Risso)	15.8	57.0
	Lepidon eques (Gunther)	20.0	81.7
Anguillidae	*Anguilla anguilla* (Linnaeus)	-	-
Soleidae	*Solea solea* (Linnaeus)	28.3	72.4
	Buglossidium luteum (Risso)	31.7	52.8
Osmeridae	*Osmerus eperlanus* (Linnaeus)	-	-
	Mallotus villosus (Muller)	24.9	92.2
Bothidae	*Scophthalmus maximus* (Linnaeus)	32.0	111.4
	Lepidorhombus whiffiagonis (Walbaum)	28.5	71.9
Lophiidae	*Lophius piscatorius* (Linnaeus)	24.9	104.2
Triglidae	*Eutrigla gumardus* (Linnaeus)	30.3	86.3
	Aspitrigla cuculus (Linnaeus)	42.4	134.2
Macrohamphosidae	*Macrohamphosus scolopax* (Linnaeus)	11.4	27.7
Cottidae	*Cottus scorpius* (Linnaeus)	19.0	47.1
	Cottus bubalis (Euphrasen)	24.9	50.9
Centrolophidae	*Centrolophus niger* (Gmelin)	11.0	27.0
Rajidae	*Raja batis* (Linnaeus)	-	130.4

Figure 25. The relative size distribution for (from top) pink oxidative fibres, pink intermediate fibres, small white fibres, large white fibres, and small diameter fibres from the dorsal fin of *Hippocampus abdominalis*.

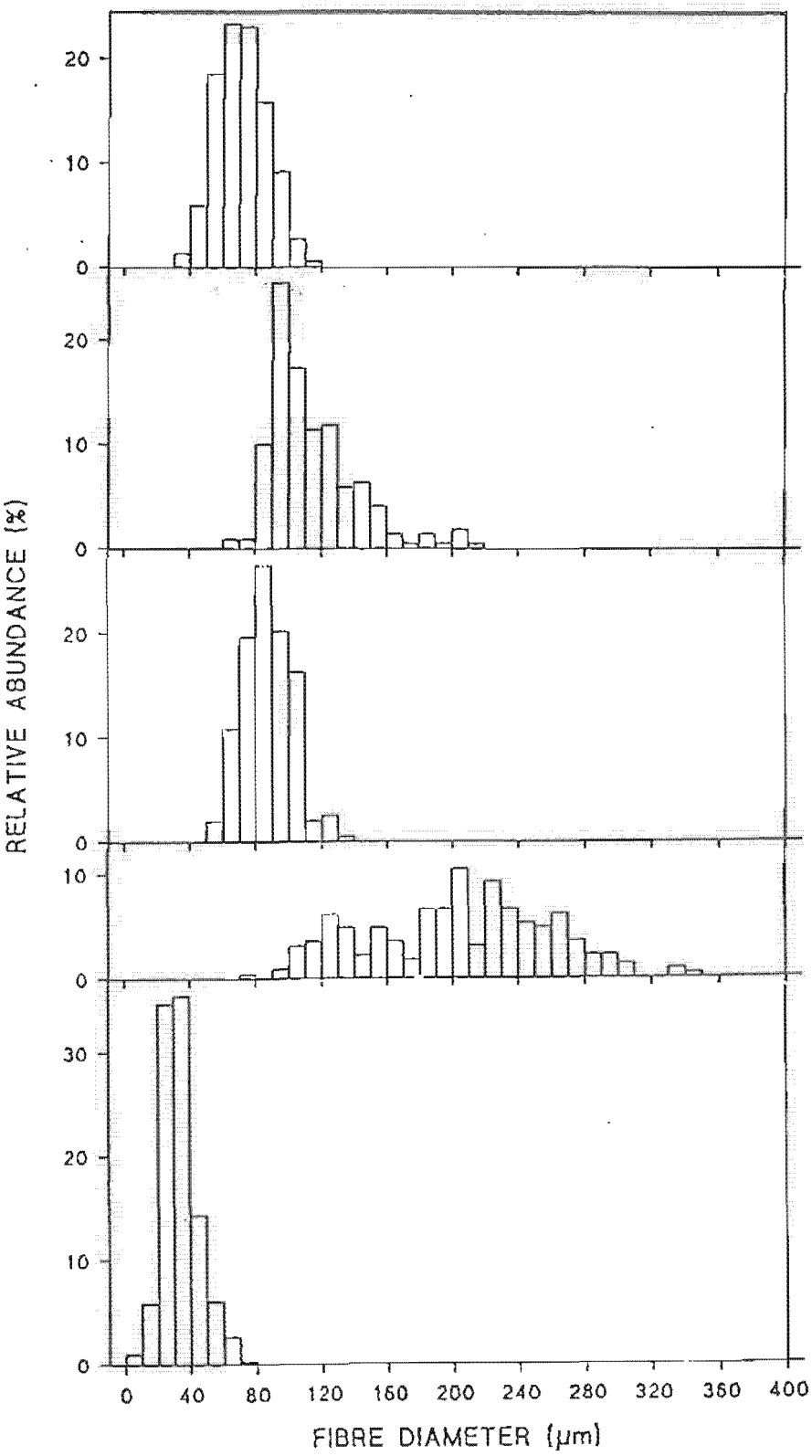
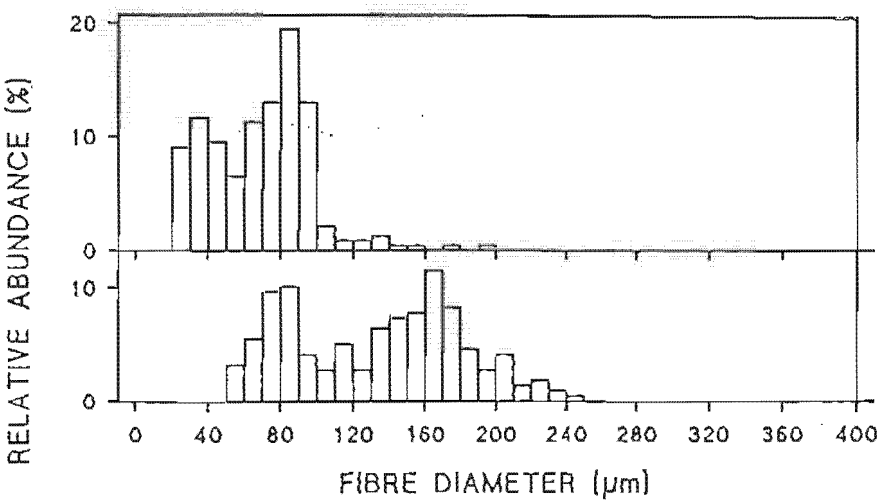
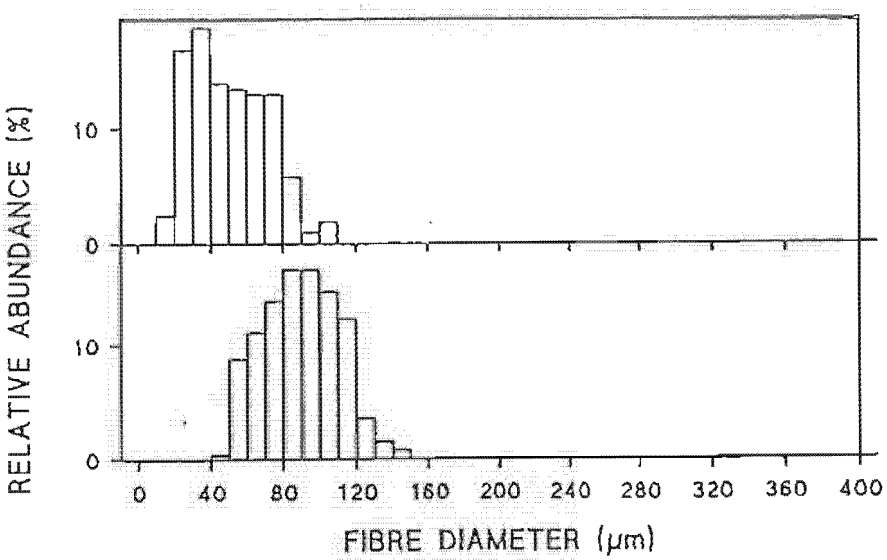

RELATIVE ABUNDANCE (%)
20
10
0
20
10
0
20
10
0
10
0
30
20
10
0
0
40
80
120
160
200
240
280
320
360
400
FIBRE DIAMETER (μm)

Figure 26a. The relative size distributions of (from top) tonic fibres, and white fibres in the mid region of the myotome of *Hippocampus abdominalis*.

Figure 26b. The relative size distributions of (from top) tonic fibres, and white fibres in the distal region of the myotome of *Hippocampus abdominalis*.


RELATIVE ABUNDANCE (%)
20
10
0
10
0
0
40
80
120
160
200
240
280
320
360
400
FIBRE DIAMETER (μm)


RELATIVE ABUNDANCE (%)
10
0
10
0
0
40
80
120
160
200
240
280
320
360
400
FIBRE DIAMETER (μm)

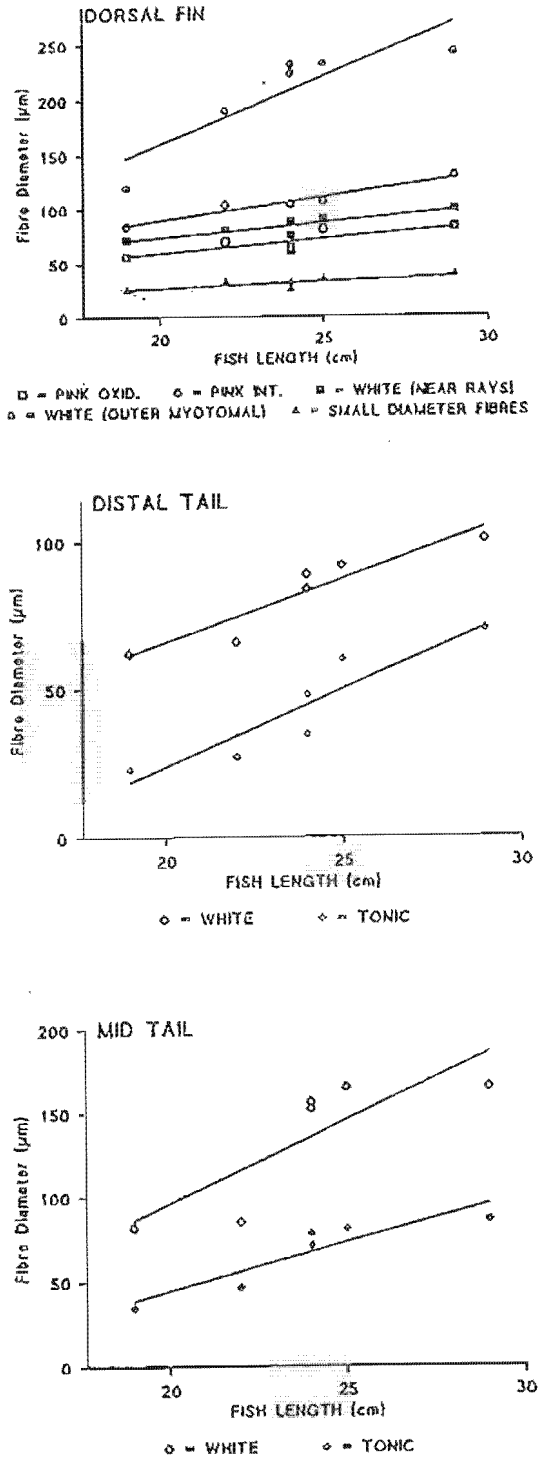


Figure 25. Fibre diameters of dorsal and myotomal fibres of *Hippocampus abdominalis* (all measurements in µm).

Discussion

The musculature of the dorsal fin

The dorsal (locomotory) musculature of the seahorse completely lacks the mATPase labile red fibres which have traditionally been implicated in a sustained role. The most oxidative fibres present stain corresponding to the intermediate (pink) muscle described in other teleosts (Johnston *et al.*, 1974).

The presence of intermediate muscle fibres was first noted by Ogata (1958a,b). In the typical scenario, pink muscle fibres have levels of oxidative and glycoltic activity intermediate between those in the red fibres and those in the white (George and Bokdwala, 1964; Ogata and Mori, 1964; Bokdwala and George, 1967a; Johnston *et al.*, 1974; Patterson *et al.*, 1975; Tatarczuch and Kilarski, 1982). This intermediary pattern is parallelled in most cases by the levels of locomotory effort at which it is usually recruited. The classic example is that of the carp, *Cyprinius carpio*, in which the pink fibres are utilised at swimming speeds just too high for the red muscle, having the effect of sparing the white muscle and avoiding the disadvantage of an oxygen debt (Johnston *et al.*, 1977). However, pink muscle varies from species to species in terms of quantity, function and cellular characteristics. Some fish possess it in comparitively large proportions; while in others such as the trout, its presence is not extensive, and indeed many fish do not possess it at all (Patterson, *et al.*, 1975).

Furthermore, pink muscle fibres have been found to be present in more

than one population in a single species. The typical pattern is that of two subpopulations with characteristically disparate myofibrillar ATPase qualities; one showing low mATPase activity after both acid and alkali pre-incubation; the second displaying a stable mATPase response after acid and alkali pre-incubation (Akster (1985: *Perca fluviatilis*; Patterson *et al.*, 1975: carp, coalfish and mullet; Mosse and Hudson, 1975; and Tulloch (1990: *Obliquichthys maryannae*).

Pink muscle that is stable to acid and alkali pre-incubation is more common in teleosts (Mascarello, 1986; Johnston *et al.*, 1974; Mosse and Hudson, 1977; Akster and Osser, 1978: van Raamsdonk *et al*, 1982). An acid- and alkali-labile mATPase activity occurs less frequently and is often present as a superficial layer only (Mascarello, 1986; Akster, 1983).

Tulloch (1990) proposed that the locomotory capacity of the mATPase stable oxidative pink fibres of *O. maryannae* was sustainable as the fish swam continuously; a finding which suggests a more distinctively red character than 'typical' pink muscle fibres. In this case, the oxidative metabolism of the aerobic pink fibres, utilising both lipid and carbohydrate, was thought to be analogous to the characteristic metabolic and functional profile of red fibres, which are ideally suited for the prolonged low intensity effort typically required of them (George 1962).

This perspective is by no means unique, as it has often been suggested that the pink muscle of certain species is similar in character and function to white or red muscle. Patterson *et al.*, (1975) recorded that the two differentiated subpopulations in the pink fibres of carp, coalfish and mullet were composed of small and large diameter fibres which were similar in terms of staining and size to red and white fibres respectively.

These results were parallelled by the pattern observed in the seahorse

dorsal oxidative musculature, in which the smaller fibres were seen to stain more darkly for oxidative enzyme activity. The extensive mitochondrial content of the oxidative pink fibres of *Hippocampus* indicates a high resistance to fatigue, and suggests that these fibres have an aerobic capacity similar to that of red muscle described in other species (Davison, 1983; Kryvi and Totland, 1978; Sanger *et al.*, 1990). However, in spite of the similarity to the results from Patterson *et al.* (1975), George and Bokdwala (1962) and Mosse and Hudson (1975), it cannot be inferred that these fibres are necessarily more similar in terms of function to red fibres than the larger pink intermediate fibres. These factors simply indicate that the intermediate pink fibres have a more glycolytic capacity, and that the levels of their oxidative enzymes are lower. Furthermore, the function of the oxidative dorsal musculature in the seahorse is unlikely to be sustainable as these fish do not swim continuously.

Whether the contractile speeds of the pink fibre subpopulations are identical cannot be determined on the basis of the histochemical and ultrastructural parameters in this study; however the differentiated mATPase staining pattern may represent a disparity. As mATPase activity is a heavy chain property, small changes in amino acid sequence could account for the observable differences in the staining characteristics between these two groups; it may also affect the speed of muscle fibre contraction.

Barany (1976) proposed that the quantity of myofibrillar ATP-ase present could be correlated with the speed of shortening of the muscle fibres. Direct biochemical measurements of myofibrillar ATP-ase activity have shown the activity to be about four times higher in the fast-contracting white fibres in comparison to the red (Johnston *et al.*, 1972; Johnston and Tota, 1974); although Nag (1972) reported a level around three times that found in red fibres. Since mATPase lability is a property characteristic of slow-contracting red fibres, it

may be possible that the intermediate (mATPase labile) pink fibres are more slowly contracting than the oxidative (mATPase stable) pink fibres. However, the fact that pink muscle *per se* usually stains the most intensely for mATPase enzyme activity does not necessarily denote that it is the fastest-contracting, in fact this is a rank assured of the white fibres (Johnston *et al.*, 1977). Furthermore, larger glycolytic fibres tend to be typified by a faster contraction speed as they rely on anaerobic metabolism. On the basis of this information, it would be hazardous to claim a reliable assessment.

The fact that mATPase stability is also indicative of white fibres is supported by immunohistochemical studies, using antisera specific for the myosins of red and white muscle (Mascarello *et al.*, 1986). Pink muscle fibres were found to give the same reaction to anti-F and anti-FHC sera as white muscle, which indicates that the isoforms present in the pink and white muscle are similar (Mascarello *et al.*, 1986). The pink muscle myosin differs from white in its heavy chain isoform, though the light chains are indistinguishable (Johnston *et al.*, 1977; Scapolo and Rowlerson, 1987). The suggestion that pink and white muscle myosins are similar is supported by the fact that carp pink muscle contains 'fast' type light chains (Johnston *et al.*, 1977).

Further evidence supporting this theory has been obtained by van Raamsdonk *et al.* (1982) and te Kronnie *et al.* (1983), who demonstrated that pink muscle fibres react with antisera specific for myofibrillar proteins of various vertebrate fast muscles. However, pink myosins are rarely observed to be similar to those of the red fibres, in which the principal myosin shows a predominantly alkali-labile mATPase activity (Rowlerson *et al.*, 1985).

Despite the results of the works cited above, it is generally accepted that pink fibres do, nonetheless, contain a distinct form of myosin (van Raamsdonk *et al*, 1982; te Kronnie *et al.*, 1983). The innovation of polyclonal antibodies

raised against fast myosin from avian muscle (van Raamsdonk *et al.*, 1982), and more recently, a monoclonal antibody raised against fish myosin and apparently specific for pink muscle (van Raamsdonk, pers. comm. to Rowlerson, 1985) has provided decisive evidence.

The fact that pink muscle myosins vary considerably may in time reveal a different perspective. The work of Carraro *et al.*, (1981) indicates that the range of variation in fish myosins might be as extensive as that seen between avian and mammalian myosins. Rowlerson *et al.*, (1985) described three types of pink muscle with regard to the types and distributions of myosin isoforms: a mosaic of red and white fibres; a transition zone between red and white muscle; and a zone consisting of one or more fibre types with an mATPase profile unlike that found in either the red or white muscle.

According to Rowlerson *et al.* (1985), none of the latter features showed any striking correlation with taxonomic groupings or lifestle, although this may be a product of the small number of fish sampled.

Similarities have been noted not only between individual fish myosins, but also between those of fish and mammals. Various studies have indicated that mammalian and piscean fast (white) muscles and slow (red) muscle myosins have a number of features in common, namely three and two light myosin chains respectively; alkali-stable and alkali-labile mATPase activity; and differentiated immunoreactivity (Connell, 1958a,b; Focant *et al.*, 1974). However, several "species-related differences" have also been reported (Focant and Huriaux, 1976; Focant *et al.*, 1976), which may or may not be the consequence of methodological vagaries. These comparitive studies are hard to accurately quantify, as the extent of diversity of myosin types and methods used makes it difficult to assess individual results, and to distinguish between effects of differences in the method used and genuine species-related variation.

Unfortunately, the literature is filled with examples which highlight the hazards of comparitive work. It cannot be stressed enough that conditions for the demonstration of investigative techniques must be carefully monitored; and if necessary, altered to suit the individual species. This consideration is well-documented in the case of mATPase analyses, in which quite small differences in the composition of pre-incubation and ATP-containing buffers may result in large differences in mATPase activity (Guth and Samaha, 1972).

Furthermore, certain aspects of the environment may alter the staining characteristics of fish muscle. Chayen *et al.*, (1987) found that seasonal differences are significant in the staining characteristics of the plaice, in which regions distinguishable in summer months could not be differentiated in winter. Nor was the intensity of stains as strong, although fibre size remained much the same from season to season. Johnston, Davison and Goldspink (1975) found that temperature acclimation also produced adaptive changes in the myofibrillar ATPase response. At 1°C, the myofibrillar ATPase activity of the cold acclimated fish was 2.8 times higher than that of a warm acclimated fish assayed at the same temperature; additionally, the myofibrils were found to have different thermostabilities. In these cases, temperature acclimation was suggested to produce either a change in the structure or accessibility of the mATPase active site.

Along with the wide range of physiological features of the pink muscle, the numerous proposed functions for these fibres have also been the topic of debate. Patterson, Johnston and Goldspink (1975) suggested that since in some species the quantity present is comparable with that of the oxidative fibres, the occurrence of pink fibres might be associated with a particular locomotory type. The original hypothesis suggested by Bokdawala (1967) proposed that the

occurrence of pink muscle was associated with the ability to swim continuously for long periods; an idea which was echoed by Patterson *et al.*, (1975). However, no pink fibres were found in the semi pelagic fish *Hemiramphus* sp, which was observed to swim continuously (Mosse and Hudson, 1975); in fact there appears to be considerable variability in the occurrence of these fibres in relation to locomotory activity. Furthermore, the overwhelming bulk of evidence has implicated the red fibres in a sustained locomotory role.

More recent results indicate that fish which do not possess red muscle, but rely instead on a comparitively large proportion of pink fibres for locomotion, tend to be characterised by a mode of life in which sustained cruising is not a feature (Mosse and Hudson, 1977).

The presence of intermediate fibres in the locomotory musculature of the seahorse supports this theory, as this fish is certainly rather inactive. Although it swims slowly, implying that its locomotion may be sustainable, the operation of the dorsal fin actually appears to require considerable expenditure of energy. Breder and Edgerton (1942) reported that the dorsal fin of *Hippocampus hudsonius* vibrated at the frequency of 35 undulatory cycles per second.

The fact that the seahorse is negatively buoyant also contributes to the workload of these delicate structures. In addition, short distance movements (which are typical of seahorse behaviour) are thought to require the recruitment of relatively fast contracting fibres to overcome inertia and gravity (Lindsey, 1978).

Since red muscle in most fish has been shown to contract slowly (Johnston *et al.*, 1977; Davison, 1983), it is unlikely that these fibres would be able to produce the fast contractions needed to produce such rapid undulation of the dorsal fin, or to overcome the inertial and gravitational forces. Pink muscle fibres, on the other hand, are typically observed to contract at speeds

intermediate between red and white fibres (Johnston *et al.*, 1977), which would make them a more suitable choice. Significantly, Bergman (1964) found that the contractile speeds of the dark muscle in the seahorse *Hippocampus hudsonius* were faster contracting than those of the white.

The metabolic properties of pink muscle also appear to constitute a superior option for the seahorse. As pink muscle typically has a lower oxidative capacity and a higher glycolytic enzyme activity, it functions partly anaerobically due to the comparitively reduced oxygen supply (Johnston *et al.*, 1977). The fact that pink muscle fatigues more rapidly than red fibres is not normally a disadvantage for the seahorse, which is usually found resting attached to seaweed, and which does not attempt to swim except when changing static position. In addition, the pink oxidative fibres contained large quantities of mitochondria, which would increase the oxidative capacity. The accumulation of lactate eventually does reduce the metabolic efficiency (Lehninger, 1982), but this disadvantage is probably outweighed by the benefits of the greater power output.

Under the electron miscroscope, the major distinction between the two populations of dorsal fin pink fibres was the differentiated levels of capillarisation and mitochondria. The pink oxidative fibres were characterised by extensive levels of these elements, while the intermediate fibres displayed a significantly reduced aerobic profile. These findings were supported by the histochemical results, in which the pink oxidative fibres appeared to contain greater quantities of lipid and oxidative enzymes. Lipid was obviously an important substrate in locomotion; however the oxidative pink fibres also contained more glycogen, another substrate commonly observed in aerobic metabolism.

During typical movement from perch to perch, the seahorse probably relies on the oxidative pink fibres, employing aerobic lipolysis to obtain ATP. Lipid is

the preferred substrate of aerobic metabolism. It is usually found in greater quantities in red muscle than in white, constituting 11 per cent in brook trout *Salvelinus fontinalis*, and plaice (*Pleuronectes platessa* (Johnston and Moon, 1980b). Accordingly lipase activity is much greater in red muscle than in white (George and Bokdawala, 1964).

Storing and metabolising lipid has two advantages. Firstly, as lipid has a lower specific gravity than water, it provides buoyancy. Distribution patterns of lipid storage are variable among teleosts; in fatty fish such as anchovies, a layer of fat cells occurs between the skin and the superficial muscle cells (Gill *et al.*, 1987); while in other fish like the mackerel (Bone, 1978b) and the eel, adipocytes are widely distributed among the white muscle fibres.

Lipid content also varies according to the portion of the body, thus it is preferentially deposited (Gill *et al.*,1987). The extent to which the muscle lipid reserves of such fish represent metabolic stores or function as part of a buoyancy mechanism is unknown, but the fact that muscle lipid contents may be higher in winter in some fish (Lahti, 1987) indicates that it may be recruited to cope with energy demands. In the channel catfish, increased lipid storage in the winter months is parallelled by elevated levels of activity in hydroxy acyl Co A dehydrogenase, an enzyme involved in fatty acid catabolism; thus it probably serves to expand aerobic metabolism capacity during these months (Johnston and Moon 1980a,b). These authors also noted increased activity of hydroxy acyl Co A dehydrogenase in the winter axial muscles of the brook trout and coalfish. Other studies have demonstrated an increase in axial muscle lipid associated with variables such as environment, feeding competition, sex and temperature acclimation (Bilinski, 1982; Lahti, 1987; Sanger *et al.*, 1987). A reduction in glycogen and increase in lipid storage may also play a role in increasing buoyancy; even if the amount of lipid is not stored it will contribute to weight

reduction.

Fontaine (1975) notes the particular importance of red muscle lipids in species which undertake long spawning migrations. Lipids tend to be found in lesser amounts in "quiet" fish such as carp and tilapia, but may be generously abundant in migratory fish such as the horse mackerel (Fontaine, 1975).

In terms of energetics, lipid is advantageous in that it has a higher energy content by weight compared with glycogen and unlike this molecule, its storage does not involve the associated storage of significant volumes of water. In energy units this means that glycogen is ten times as expensive to store than lipid for the same return (Alberts *et al.*, 1983). Furthermore, aerobic glycolysis yields just 38 mols of ATP per glycosan unit, whereas aerobic lipolysis yields over 100 (Hill and Wyse, 1989). Obviously, lipid is a much more efficient fuel.

The disadvantage of using lipid as an aerobic substrate is that it is only suitable for slow sustained locomotion, hence its abundance in the red fibres. Although both aerobic lipolysis and glycolysis require oxygen, lipolysis is a much slower process than glycolysis, as glycogen is much quicker to mobilise.

When increased energy expenditure is required, for example when the seahorse is forced to move long distances or enlist maximum power in escaping a predator, the recruitment of glycogen as a substrate in the oxidative pink fibres probably provides an immediate source of energy.

In thepink oxidative fibres, a compromise has probably been reached between relatively low chemical efficiency for high-speed machinery, and a highly efficient system used for continuous operation.

The lack of mitochondria, lipid and oxidative enzymes in the intermediate pink muscle indicates that anaerobic glycolysis is employed in these fibres, presumably only when the oxidative capacities of the small pink fibres are

exhausted.

Although glycogen is the primary substrate for anaerobic metabolism, it is reported to be present in higher concentrations in red muscle than white (Bone, 1966; Wittenberger, 1968; Johnston and Goldspink, 1973). Johnston (1981a) suggested that as the red fibres have a relatively small volume compared to the white, a proportionally greater quantity of glycogen may be used in the white muscles during fast swimming. However the anomaly can probably best be explained by the fact that glycogen is the stored form of glucose, which is the energy substrate. Thus the observation that the pink intermediate fibres stain more lightly for glycogen actually represents an indicator that it is constantly being recruited as a source of energy.

This trend also confirms that lipid is the more important substrate in the oxidative pink fibres, since the glycogen staining pattern indicates that the molecule is generally not mobilised. Furthermore, since the fast white fibres stain the most lightly for glycogen, they are undoubtedly depleting their stored reserves to the greatest extent.

Biochemical determinations of glycogen concentrations vary between individual species, and have been shown to fluctuate enormously depending on activity level and nutritional status (Love, 1970). Brown trout build up large intracellular stores of glycogen in fibres recruited during training (Davison and Goldspink, 1977); whereas a shift to lipid metabolism following exercise training was proposed to occur in the coalfish, *Pollachius virens* (Johnston and Moon, 1980a, b). These results were supported by biochemical analyses of metabolite stores and enzyme activities (Johnston and Moon, 1980a,b).

Recruitment of the intermediate pink fibres enables an increase in power output while conserving energy of the white fibres. In exceptional circumstances however, it is possible that the anaerobic white fibres are recruited for

locomotion when the energy reserves of the pink fibres are drained. As these fibres contain the least numbers of mitochondria, they represent the most inefficient way of gaining ATP for driving the actin-myosin interaction. However, the superior contractile forces of these glycolytic fibres enables potentially greater locomotory speeds to be reached; an advantage which is maximised by their large numbers, size, and position-situated at the point of greatest distance from the dorsal rays (the fulcrum). This location allows the exploitation of maximum torque during locomotion and hence optimum power.

The location of the pink glycolytic fibres also enables exploitation of the above effect, to a lesser extent. Although they cannot attain the forces of contraction of the outermost muscle, the fact that they lie further from the fulcrum than the oxidative, lipid-rich oxidative pink fibres indicates that the maximum power obtainable would undoubtedly be superior.

A similar fibre distribution pattern is observed in both the locomotory fins and the myotomes of other, more typical piscean forms, in which a graduation from the slowest-contracting, most oxidative fibres through to large fast-contracting glycolytic muscle can be observed (Lindsey, 1978).

Small diameter fibres, located adjacent to the dorsal fin rays, were not thought to play any role in locomotion as they were present in such small numbers. Their most plausible function in the locomotory musculature is that of a postural nature.

These fibres were of a regular size and shape. They were always closely associated with a subpopulation of white fibres of less than typical dimension or size variability, (although their staining characteristics were certainly comparable).

The dorsal small diameter fibres may be recruited when the dorsal fin is not in active use. During periods of quiescence, the fin lies flat against the body in

a state of relaxation. However, at other times such as brief intermissions in swimming, the fin is held erect. It is at these moments that the small diameter fibres, possibly in association with the smaller white fibres (which may support these fibres both structurally and functionally), probably gain useful employment. The maintenance of an erect dorsal fin may be important in terms of camouflage tactics such as immobility, which are essential in successful predator avoidance and prey capture in these fish.

The charateristics of small diameter (tonic) fibres will be discussed in full in the next section.

The Myotomal Musculature

The fact that no staining for lipid or oxidative enzymes whatsoever was observed in the white fibres, in either the myotome or the dorsal fin, reflects the fact that these fibres function entirely anaerobically.

The white fibres probably play very little part in the myotome. They are clearly not recruited for high-speed swimming, or in fact any sort of locomotion. Their only probable functional roles would be to execute the lashing movements seen in predator escape responses when the seahorse is seized; and possibly also to effect a strong grip on the substrate to prevent its displacement during these circumstances. It is unlikely that the tonic fibres could be capable of such vigorous or powerful function (even though they are present in significantly larger numbers in this fish), as tonic typically have a very slow speed of contraction (Davison and MacDonald, 1985).

The lack of mitochondria in the large white fibres indicates that they function by anaerobic glycolysis. This suggestion is supported by their stable mATPase enzyme activity; the presence of glycogen observed in the ultrastructural analyses; and the lack of staining for either lipids, the preferred aerobic substrate, or oxidative enzyme activity. Although anaerobic glycolysis is a relatively inefficient way of gaining ATP for driving the actin-myosin interaction, it is nonetheless a suitable energy pathway for the white fibres of the seahorse as they are probably recruited fairly rarely for short periods of time.

A significant population of small diameter (tonic) fibres was also observed in the seahorse myotome. These fibres are typically present in very small numbers in teleosts, and are very slow contracting with a poorly developed

sarcoplasmic reticulum (Davison and MacDonald, 1985). In view of these characteristics, tonic fibres are not thought to be involved in locomotion. Recent evidence has suggested that they may be analogous to the tonic fibres of mammals, which are thought to play a role in maintaining posture (Davison and MacDonald, 1985; Johnston, 1981; te Kronnie *et al.*, 1983; Morgan and Proske, 1984).

In the seahorse, the myotome has been modified into a prehensile anchor and plays no propulsive role in swimming, although it is thought to act as a balancing aid when the animal is not attached, as also noted by Bergman (1964) and Breder and Edgerton (1942). However, the predominant role of the tail is to anchor the fish as it feeds; a function which may be described as postural. Since tonic fibres are present in large numbers in the myotome of the seahorse, it would appear that that the results of this study support the theory that tonic fibres of teleost fish are in fact analogous to the tonic fibres of mammals, at least in this animal.

The myotomal tonic fibres in the seahorse are thought to play a role not only in holding the tail of the fish in position, but also in bending the tail around the seaweed substrate. Although these fibres have not been implicated in a role other than purely postural to date, the observation that they are present in the myotome of the seahorse in such high proportions suggests that they probably are capable of performing this function. The fact that tonic fibres are thought to contract very slowly would be advantageous in that the operation of this function would not require the expenditure of large amounts of energy.

Further evidence implicating the tonic rather than the white fibres in a postural role is provided by the observation that the seahorse spends most of its time attached to a convenient hold by its tail. In considering the fact that the tail never seems to fatigue, the maintenance of the animal's grip is far more likely

to be effected by the tonic fibres, which are conveniently located at points of contact on the underside of the myotome, than by the white fibres which fatigue quickly at great energetic cost.

The tonic fibres found in the myotome were of a far more variable shape and size in comparision to those in the dorsal fin. Myotomal tonic fibres also contrasted with their dorsal counterparts in that they stained slightly for oxidative enzyme activity, whereas dorsal SDF did not. The differential staining characteristics have rarely been noted in the same fish to date, although inter-specific differences have often been observed; Johnston *et al* (1974) found that SDF were mATPase stable following alkali pre-incubation, while (Kilarski (1990) and Davison and McDonald (1985) found that they were not. The pattern found in the seahorse indicates that the tonic fibres were of two heterogenous populations, each probably playing a different role.

The existence of tonic fibres (also known as small diameter fibres, or SDF) in the myotomes of fish which do not employ this structure in locomotion is not unique. According to Johnston *et al.*, (1975); fishes which employ the carangiform mode of swimming, such as the trout, tend not to have SDF in their locomotory musculature. In contrast, the carp, which does not employ a myotomal method of swimming, has a small population of these fibres in this part of its' musculature (Johnston *et al.*, 1974); and many SDF are found in the trunk musculature of the bully, which is held rigid during swimming (Davison, 1983a).

Small diameter fibres have been recorded from both elasmobranchs (Bone, 1966) and teleosts (Johnston *et al.*, 1974; Patterson *et al.*, 1975; Carpene *et al.*, 1982). These fibres are always associated with the red muscle but are located in

different regions in different species. In fish myotomes, a gradient can often be seen in the intensity of metabolite and enzyme activity staining from the most peripheral red fibres to deep white fibres (Davison, 1985; Davison and Goldspink, 1984; Patterson *et al.*, 1975). The same gradient is also observed in other teleosts, with the exception that small diameter fibres are located in the region traditionally occupied by red fibres (Davison, 1983a, 1987).

In Davison's (1983a) study, small diameter fibres occupied the position usually populated by slow oxidative muscle, which was found to be absent in the lateral musculature of this fish. When swum in a Blazka-type water tunnel, the bully remained on the bottom of the respirometer, and if forced to swim it rapidly fatigued, demonstrating the lack of aerobic musculature which would have effected sustained locomotion (Davison, 1983a). The same locomotory reluctance was observed in the seahorse when it was forced to swim around the tank.

Tonic fibres tend to be limited to the periphery of the myotome only, most frequently being found just under the skin, deposited in a single layer among the red fibres (Kilarski and Koslowska, 1985). This type of localisation is typical of small fast-swimming fish like *Brachydanio rerio* (van Raamsdonk *et al.*, 1980), and *Gasterosteus aculeatus* (te Kronnie *et al.*, 1983). Starling (1985) found that small diameter fibres were scattered throught the myotome of the spotty *Pseudolabrus celidotus* rather than being grouped as found in the myotomes of other fish studied (Walesby *et al.*, 1982; Davison 1983a).

Tonic fibres may also be found scattered among the red fibres, as in the myotome of *Esox lucius* (Zawadowska and Kilarski, 1985); or they may form a belt at the boundary between the intermediate and slow fibres (Kilarski and Zawadowska, 1985: *Cyprinius carpio* and *Carassius auratus*).

The fact that small diameter fibres often occur in zones of transition to

progressively larger fibres has indicated that they may be regions of continuing growth. It has been shown that the number of SDF increases during growth in the cod *Gadus morhua* (Walker, 1970). The 'growth theory' proponents suggest that the tonic fibres are in fact developing red fibres; a consensus which conveniently ignores all evidence to the contrary. It seems rather questionable that a developing fibre would vary so much from the parent population, as small diameter fibres do not contain the large numbers of mitochondria and capillaries observed in red fibres. Furthermore, tonic fibres are sometimes found in high numbers in very large fish in which it would be expected that fibre recruitment would have been completed (Weatherley and Gill, 1981).

How the differential morphological and staining characteristics between 'growing' and 'mature' fibres, lack of transitional staining between adjacent populations, and presence of many SDF (in some cases *more*) in fully grown fish came to be regarded as evidence that small diameter fibres are a growth stage of red fibres is a topic for debate in itself! The theory is certainly deserving of some suspicion. In fact, it seems most likely that tonic fibres are a separate group in their own right with respectively individual properties.

Since the number and size of small diameter fibres increases with size in some fish (Davison 1983a), it is unlikely that small diameter fibres are degenerating fibres. However, the fact that some fish have been found where the SDF had been partially or totally replaced with connective tissue puts some doubt on this issue.

Ultrastructure of the musculature

The most informative component of muscle investigative studies is generally the ultrastructural category. In addition to providing invaluable assistance in fibre type confirmation, ultrastructural examination of the different fibre types is essential in order to enable adequate conclusions of the respective functional roles to be reached.

In recent years, a growing number of papers have addressed the topic of fish muscle ultrastructure, both descriptively and quantitatively. However, the comparitive appraisal of such studies should be viewed with some trepidation, as fish muscle ultrastructure is affected by several influential factors. For example, although slow myotomal muscles have been reported to comprise a fairly homogeneous population in brook trout (Johnston and Moon, 1980a), eels (Egginton and Johnston, 1981a,b), and anchovies (Johnston, 1982b); this pattern is not observed in tench, and presumably some other species (Johnston and Bernard, 1982a,b). Fast fibres often display an even more significant heterogeneity, not only with respect to size (Johnston and Moon, 1981); but also according to regional distribution in the myotome (Johnston and Bernard, 1982b). Furthermore, factors such as endurance exercise training (Johnston and Moon, 1980a), oxygen availability (Johnston and Bernard, 1982a,b), starvation (Patterson and Goldspink, 1973), and environmental temperature (Johnston and Maitland, 1980) can also markedly affect muscle ultrastructure. These factors probably account for at least a small percentage of the variation observed between different studies of fish muscle ultrastructure.

According to Quaglia, (1980), Franzini-Armstrong and Porter (1984),

Bishop and Odense, (1967); Kilarski, (1967); and Nag (1972); red and white fibre types do not differ in terms of their ultrastructure, despite the fact that they are quite different histochemically (Patterson *et al.*, 1975; Kryvi and Totland, 1977), physiologically (Johnston *et al.*, 1977; Mosse and Hudson, 1977), and morphometrically (Patterson and Goldspink, 1972; Kryvi, 1977). However, ultrastructural differences between fibre types have recently been quantified in a number of studies (Akster, 1985; Davison, 1983a; Kryvi and Totland, 1978; Sänger *et al.*, 1990). According to Shindo *et al.* (1986), myofibrillar packing is usually more regular in white muscle fibres in comparison to red; and in terms of myofibril arrangement, white fibres are sometimes observed to be elliptical or nearly circular at the central area of muscle fibres, whereas red fibres tend to be more asymmetric at the circumference (Shindo *et al.*, 1986). Small muscle fibrils may also be arranged in a pattern resembling a cartwheel, with spokes radiating to a small central hub of sarcoplasm. This feature is not observed in elasmobranchs, holocephali or higher vertebrates. It may be related to a pattern of fibre growth in teleosts (Shindo *et al.*, 1986).

Other differences between the fast and slow fibres of teleosts have also been recorded, and will be discussed in this section.

The ultrastructure of the seahorse white fibres is not unusual. The myofibrils in the white muscle were arranged in a closely packed, regular pattern. Myofibrillar packing is usually more regular and dense in fast glycolytic fibres than in slow fibres, reflecting the requirement of this fibre type to develop tension extremely rapidly during burst swimming (Webb and Weihs, 1983).

The sarcoplasmic reticulum in these fibres was well developed, a feature also associated with the fast contraction speeds of these fibres.

Differences in the extent and development of the sarcoplasmic reticulum

may or may not be observed between the two primary fibre types. The surface of the whole sarcoplasmic reticulum is responsible for the re-uptake of calcium, which brings about the relaxation of the muscle fibre (Winegrad, 1970). Quaglia (1980) found that the white and red myofibrils of the grey mullet were surrounded by an equally developed sarcoplasmic reticulum, consisting of a fenestrated collar in the H band region, several longitudinal tubules in the A-I band regions, and terminal cisternae always located at the Z disc.

In contrast to the latter results, Nag (1972) reported different sarcoplasmic surface areas in the muscles of *Salmo gairdneri*, in which a relatively larger sarcotubular system was observed in the fast white fibres compared to the red. Differences in the extent and development of the sarcoplasmic reticulum are suggested to be related specifically to the speed of contraction (Nag, 1972). Porter (1961) suggested that highly developed longitudinal sarcoplasmic reticulum is found in fast-acting muscles and may be related to an active role in the return of the fibre to its relaxed state. However, according to Akster (1985), the large differences in relaxation times between the fibre types of the perch were thought to be due to a difference in biochemical properties of the systems that eliminate calcium, and not to a difference in surface area of the sarcoplasmic reticulum.

The fact that fast fibres typically display numerous chemical differences in comparison to slow fibres provides support for this suggestion. Fast fibres display higher activities of low molecular weight calcium-activated ion binding protein relative to slow fibres (Harrison and Miller, 1981; McArdle and Johnston, 1981). According to Lindsey (1978), it is possible that these low molecular weight proteins may be concerned in regulating muscle contraction velocity, perhaps by their buffering effects on calcium-activated ATP-ase. Penney and Goldspink (1980) reported that the white fibres of the axial muscle in temperature-adapted

and control groups of carp *Carassius auratus* differed in the relative areas of the SR, but not terms of the rate of calcium uptake, indicating that biochemical elements are tightly regulated in fish muscle.

In mammalian muscle, changes in twitch characteristics brought about by long term electro-stimulation are accompanied by differences in the polypeptide composition of the sarcoplasmic reticulum, and in parvalbumin content (Heilmann and Pette, 1979; Klug *et al*, 1983). Parvalbumin, which acts as a calcium shuttle between the myofibrils and the SR (Gerday and Gillis, 1976; Gillis *et al*., 1982), is present in higher concentrations in the fast twitch muscle in comparision to the slow twitch (Hamoir *et al*., 1981; Focant *et al*., 1982).

These results support the suggestion quoted by Akster (1985); that differences in fibre contraction speeds are due principally to biochemical rather than structural factors. Since the ultrastructure of red and white muscle fibres is seldom differentiated, this would indicate that at least in these individual species, biochemical composition is the definative factor in muscle contractile profiles. However, the fact that structural differences are sometimes seen indicates that biochemical factors are certainly not exclusively influential.

According to Davison (1983), pink fibres are intermediate between red and white fibres in terms of myofibrillar packing. In the seahorse, both the pink and white fibre myofibrils were observed to be arranged in long ribbons at the longitudinal orientation; an arrangement typical of both white and pink fibres in most fish (Johnston, 1980). However, in contrast to the typical pattern observed at the longitudinal orientation, observations of pink fibres at the transverse orientation were radically different from those of white fibres. Instead of being tightly packed, pink muscle myofibrils tended to be very loosely deposited within the fibre; which was also characterised by vast areas of cytoplasm.

The functional significance of this differentiation is unknown.

The pink muscle myofibrils were well defined, with a well-developed sarcoplasmic reticulum. Large distances between the sarcolemma and the contractile apparatus were also observed. These observations suggest that a highly organised route for the inward spread of the muscle action currents is present in these fibres (Bergman, 1964).

The two subpopulations of pink fibres were seemingly identical in terms of basic ultrastructure. There was a hexagonal arrangement of the thick and thin filaments in the body of each myofibril; an arrangement similar to the typical pattern seen in striated muscle of other vertebrates (Bergman, 1964).

The only significant difference between the two populations of pink fibres was the extent of mitochondrial density. Logically, the large surface area of cytoplasm typical of both subpopulations was occupied to a far greater extent by mitochondria in the oxidative fibres. Glycogen granules were evident in both types, supporting the results obtained from the histochemistry section which indicated that glycogen is an important source of energy.

The T-systems of the two pink fibre types were well-appointed and similar. The high level of sarcotubular development evident in both populations of pink fibres is typical of fish species (Johnston, 1983). However, differences in the extent and disposition of junctions between transverse tubules and the sarcoplasmic reticulum are often noted (Franzini-Armstrong *et al.*, 1987). They are related to the rate of calcium ion uptake. In white fibres, a significant calcium uptake is necessary to maintain the high frequency of contraction-relaxation cycles observed in burst swimming (Johnston, 1983).

In the seahorse, the T-tubule system of the pink fibres was more extensive than that of the white fibres. This pattern is a reversal of the typical piscean trend, according to Johnston (1983); who noted that the T-tubule system was

better developed in white muscle than in red. Although Johnstons' (1983) observation is logical given that white fibres are observed to contract at faster speeds; in the case of the seahorse, a different scenario may be evident.

Bergman (1964) conducted electromyographic analyses of the muscle fibre contractions of seahorse muscle fibres, and recorded that the pink fibres exhibited faster speeds of contraction. This pattern is not entirely an isolated occurrence; in perch *(Perca fluviatilis)* (Akster, *et al.*, 1985) and carp (Granzier *et al*, 1983); red muscle fibres generate maximum tensions that are comparable to the white fibres of the same species. However, apart from the seahorse, no examples of oxidative muscle contraction speeds *exceeding* those of the white fibres have been recorded. This hitherto unrecorded pattern may explain the unusual relationship between fibre type and extent of T-tubule development in the seahorse. However, it affords no clues as to why the triads of the sarcotubular system were located at the Z-line in both the intermediate and oxidative pink fibres, and at the A-I junction in the fast and tonic fibres.

In most teleost myotomal muscles studied, the triads are located at the position of the Z disc (Nag, 1972; Shindo *et al.*, 1986). A similar location of the tubular system is found in lampreys (Teravainen, 1971), urodeles (Totland, 1976), and anurans (Peachey, 1965). However, in fish extraocular (Kilarski, 1966), swim bladder and drum muscles (Eichelberg, 1976) and in hagfish (Korneliussen and Nicolaysen, 1973), the T-tubules are situated at the position of the A-I boundary. This is also the typical position for the triads of all mammalian muscles.

The functional significance of the differentiated positioning of the triads is unknown. Differences in the position of the triads in the seahorse are clearly not related to the energy pathway taken, as the triads of the glycolytic pink fibres were not located at the A-I junction, as in the case of the glycolytic fast fibres.

They may be be related to the actual functions of the muscles as proposed by Eichelberg (1976), who suggested that triads located at the A-I junction were more typical of non-locomotory muscles in teleosts. This proposal is supported by the results obtained in this study, in which triads were located at the z-line in the locomotory fibres (pink) and at the A-I band in the myotome (white and tonic). Alternatively, the positioning of the triads may be a consequence of the factors determining the pattern of myofibril organisation.

The Z-lines did not appear to be thicker in pink fibres than in any of the other fibre types. Pink fibres with thicker Z-lines than white have been described in some fish (Akster, 1981, 1985; Johnston, 1983; te Kronnie et al., 1983). The functional significance of this is also unknown.

The tonic fibres in the myotome of the seahorse proved to be most interesting. According to Kilarski and Koslowska (1986), the organisation of the contractile system in tonic muscle fibres is unique and reflects their capacity for sustained force generation in supporting tonicity of relaxed twitch muscle systems. The tonic fibres have twice as much protein represented by the filamentous system compared to the twitch fibres (Kilarski and Koslowska, 1986). These authors also reported that the tonic fibres have no M-line and a thick jagged Z-line (1986); and that the triad is always located at the A-I junction (1984). Furthermore, tonic fibres are typically not well delineated by sarcoplasmic reticulum, which is rather scarce and irregularly organised; a fact which further supports the non-locomotory postural hypothesis (Davison, 1983a).

Kilarski and Koslowska's extensive (1984, 1986) surveys led them to conclude that fish tonic muscles are in fact morphologically similar from species to species. However, in other studies, many lines of divergence have been

observed (for review see Hess 1970). The seahorse certainly does not conform to the prescribed profile of tonic fibres. Some of the 'typical' ultrastructural characteristics of tonic fibres described by Kilarski and Koslowska (1984, 1986) were not observed; for example, the M-line was present, although the Z-line was observed to be slightly more jagged than that of the other fibres (this may be an artifact of the preparation).

The sarcoplasmic reticulum of the tonic fibres appeared to be quite well developed; certainly similar to the white fibres. The fact that tonic fibres are typically characterised by a poorly developed sarcoplasmic reticulum has been taken as an indication that they have a slow rate of contraction (Davison, 1983a). However, since the sarcoplasmic reticulum of the seahorse tonic fibres is quite well developed, it could tentatively be suggested that these fibres may contract at a higher rate, although this suggestion cannot be quantified without the appropriate analyses. Also, the nature of the proposed function of the tonic fibres tends to cast some doubts on this idea.

Mitochondria of the oxidative pink fibres were seen in large numbers, distributed throughout the cells. In *Hippocampus abdominalis*, they surrounded the myofibrils and were even present within them, although they were found only in the peripheral sarcoplasm in *Hippocampus hudsonius* (Bergman, 1964a). The former distribution appears to be more typical of oxidative muscle: Shindo *et al.* (1986) recorded this pattern in eight fish species, whereas they were located only around myofibrils in white muscle.

In the seahorse, mitochondria tend to orient specifically at the region of the A-I band of the white and SDF fibres. In the pink fibres however, they are oriented at the level of the Z-line. In other words, mitochondria tend to be situated at the respective positions of the triads.

In terms of energetics it is logical that the mitochondria preferentially occupy this site in the sarcoplasm, as the location of the triad represents the point at which sarcolemmal depolarisation occurs; and transmission of the spread of excitation to the sarcoplasmic reticulum is effected by the enzymes of beta-oxidation which are located in the mitochondrial matrix.

In the oxidative fibres, large numbers of peripheral mitochondria were also observed, especially in close proximity to nuclei and arterioles. Muller (1976) proposed that peripheral mitochondria mainly supply energy for the active transport of metabolites across the sarcolemma, whereas the central mitochondria which surround the myofibrils supply energy for contraction.

Harrison and Miller (1984) suggested that mitochondria make a substantial contribution to calcium uptake during relaxation in cardiac muscle. However such a contribution is unlikely to be large in fish skeletal muscle because even the slow skeletal muscle fibres contain vast amounts of parvalbumin (the calcium shuttle) compared to cardiac muscle (Hamoir *et al.*, 1981; Gerday, 1982). Also, the relative volume of mitochondria is small in some slow fibres of fish. Subsarcolemmal mitochondria are likely to be of even less importance in calcium uptake during muscle relaxation (Akster, *et al.*, 1985).

Peripheral mitochondria have also been suggested to be indicative of a certain locomotory type. In the chimaera (*C. monstrosa*), as much as half the mitochondria are peripheral, located close to the sarcolemna. Deeper, larger fibres of both white and red contain little energy reserves or mitochondria, indicating that locomotory movements are effected by brief anaerobic contractions.

White muscle cells demonstrate low oxidative enzyme activity and contain few mitochondria because ATP is used faster than aerobic metabolism can provide it without compromising development of tension (Goldspink, 1980;

Johnston, 1980). Furthermore, if mitochondria are present, they take up space in the cell that could otherwise be occupied by muscle; a disadvantage for these fibres since power output is proportional to the total contractile mass recruited (Lindsey, 1978).

The mitochondria in the pink fibres of the seahorse are well developed to aid in metabolism, in comparison to those of the white fibres, which are also smaller. The size of tonic muscle fibre mitochondria was small in comparision to both kinds of twitch mitochondria, although this may be a consequence of the smaller actual size of the muscle cells.

The pink muscle mitochondria were more globular in shape that those of the white muscle, which were characteristically elongated (a pattern also noted by Love, 1980). The surface area of inner mitochondrial membranes per unit volume of mitochondrion was observed (although not quantified) to be greater in the pink fibres of the seahorse; a feature which reflects their aerobic metabolic properties. This differentiated pattern is also seen in other fish. In the very active skipjack tuna, the mitochondria in superficial red and white muscle differ from those of the deep red fibres in showing a reduction in cristae development and the presence of lamellar figures interrupting the cristae array (Bone, 1978b).

The total surface area of the mitochondrial cristae in the tonic fibres did not appear to be different from that of the twitch fibres; another distinction not generally observed in tonic fibres, according to Kilarski and Koslowska (1985).

Oxidative muscle mitochondria are generally observed to be more metabolically active; a suggestion which is probably true of the seahorse, given the functional differences of the white and pink fibres. Studies of pelagic fishes indicate that mitochondria from deep-seated red fibres consume three times as

much oxygen as those from white when the complete electron chain is operative (Modigh and Tota, 1975).

In correspondence with the larger numbers of mitochondria observed in the oxidative pink fibres, vascularisation in these fibres was more extensive. The fact that vascularisation is far more extensive in aerobic muscle has been well-documented. Dark muscle requires a good supply of oxygen-carrying blood with which to operate the aerobic musculature. Gordon (1968) found that isolated dark muscle from *Katsuwonas pelamis* consumes six times as much oxygen as the white muscle, while Stevens (1968) calculated that 2.6 times as much blood was found in the red muscles compared to the white in *Salmo gairdneri.* Love (1970) stated that dark muscle recieves about ten times as much blood as white.

The elevated blood flow in aerobic muscles not only confers a superior quota of blood to the tissues, but also faster clearance of metabolites. Johnston and Goldspink (1973b) noted that the lactate levels in the white muscle of *Carassius auratus* took up to eight hours to fall to resting levels after exercise, in contrast to red muscle which took just thirty minutes; clearly, the disparity is attributable to circulatory influences. The fact that high levels of vascularisation is associated with sustained aerobic activity is parallelled by the high levels of oxygen-storing pigments important for prolonged activity found in such muscle (Bone, 1978).

Blood flow to the capillaries is generally thought to be governed by the number of capillaries supplying each muscle cell (Johnston, 1980; Mosse, 1978). However, recent studies on exercise and recovery in trout and grayling have indicated that blood can be shunted away to other regions of the body; often preferentially to the red muscles (Neumann *et al.*, 1983; Vyazovoy *et al.*, 1982). This indicates that local control must be possible (Mosse, 1980).

While the function of the capillary network is to bring about exchanges of

metabolites between the capillary lumen and the surrounding tissues; the primary importance of the arterioles is to regulate the pressure levels of the blood entering the capillary bed (Davison, 1987).

The arterioles were surrounded by a layer of smooth muscle. In comparision to mammalian muscle, the smooth muscle layer surrounding the endothelium of teleost fishes contains very little contractile material (Gabella, 1983). This is possibly due to the differences in systemic pressures between fish and other vertebrates, as fish have a single circuit system with relatively low pressures observed within the vessels (Satchell, 1971).

The diameter of the lumen of arterioles is controlled by three mechanisms; catecholamines released by the endothelial cells; circularly oriented smooth muscle of the tunica media; and longitudinally arranged microfilaments within the endothelial cells (Davison, 1987). Higher control via the nervous system is also thought to be possible, as certain substances present locally appear to be able to control the vasodilatory response (Davison, 1987).

Pinocytotic vesicles in the walls of the arterioles and capillaries were extensive, providing a means of metabolite transport. This function is also thought to characteristic of the numerous caveolae which were present not only in the walls of the blood vessels, but also all along the muscle cells.

The fact that venules were much larger than arterioles and capillaries probably accounts for the fact that the blood pressure returning to the heart is not under tight control as in arterioles.

Nerves were often seen in the extracellular space. The high frequency with which the nerve endplates were found indicates that the pre-synaptic element is markedly elongated and parallel with the long axis of the muscle fibre.

The vast fields of collagen observed in the seahorse musculature impart increased strength and elasticity to the myotome. These connective fibrils are also important in the mechanical transmission of the forces generated by the contraction of the muscle cells, as individual cells do not usually extend from one end of a muscle to another.

The extensive area occupied by the connective tissue is undoubtedly a consequence of the unique seahorse myotome. In a typical fish employing carangiform locomotion, the power needed for contraction is provided by the myotomal muscles themselves; therefore space which could be occupied by myofibrils is not compromised by collagen to the extent seen in the seahorse. However, since the function of the seahorse myotome is not of locomotion but of maintaining a hold on the substrate, it is advantageous that a proportionally greater area is occupied by collagen. Associated with these fields of connective tissue were numerous fibroblasts, the cells responsible for the synthesis of collagen.

Alternative functions of different muscle fibre types

The literature published to date this century has provided a wealth of evidence implicating the fast and slow muscle fibre types in differentiated roles- transient burst activity and sustained speed swimming respectively. In recent years however, considerable evidence to the contrary has to a certain extent eroded the exclusive status of these labels.

Electromyographic methods of recording the functional activity of skeletal musculature demonstrate that the red muscle is active primarily at low and moderate speeds; whereas the white muscles are involved most intensively at high speeds; less at moderate speeds and only slightly at slow speeds (Davison, 1987). This generalisation is broadly true. However Johnston, (1981b) warns that there is not necessarily a simple division of labour in the fast muscle fibres, which may *not* simply be reserved for burst activity. Histochemical evidence for this has been presented for a number of different species: Johnston and Goldspink (1973: coalfish *Pollachius virens*); Johnston *et al.*, (1977: carp *Cyprinius carpio*) and Bone *et al.*, (1978).

According to Smit *et al.*, (1971), Johnston and Goldspink, (1973a,b) and Greer Walker and Pull (1973); a certain proportion of the white muscle also functions at cruising speeds in some species. Multiply innervated white fibres of some teleosts such as carp (*Cyprinius carpo*) mackerel (*Scomberi japonicus*) and brook trout (*Salvelinus fontinalis*) display graded recruitment at various speeds including low-sustainable through burst swimming (Greer-Walker and Pull, 1973; Hudson, 1973; Bone *et al.*, 1978; Roberts and Graham, 1979; Johnston and Moon, 1980a,b). In the Baikal black grayling *Thymallus arcticus,* both the red and white skeletal muscles were observed to participate in the act

of swimming throughout the range of swimming speeds investigated, although the dynamics of the two muscle types were found to be typically different (Divert and Matyukhin, 1982).

Bone *et al.* (1978) noted that the carp displayed low-level electrical activity in its white muscles at even the slowest swimming speeds at which the fish could be persuaded to move. At higher speeds, bursts of action potentials of the usual kind were observed. The reasons for this are not yet fully understood (Bone, 1982) but seem to be related to the unusual innervation patterns of the fast fibres in these teleosts.

Additionally, Weatherly *et al.* (1972) have proposed a considerably greater functional involvement of the white (mosaic) muscle of rainbow trout at slower swimming speeds than has frequently been considered possible.

In certain active pelagic species, a proportion of the white muscle has also been implicated in high speed cruising. In these cases, the white muscle uses both lipids and oxidative metabolism in addition to glycolysis. It has been suggested that both the red and white muscles of the pelagic tunas (Scombridae) have a significant capacity of aerobic glucose utilisation (Guppy, Hulbert and Hochachka, 1979). Mosse (1979) reported that white muscle in pelagic teleosts displays positive staining for enzymes of aerobic metabolism as well as the presence of extensive vascularisation and relatively abundant mitochondria. These characteristics suggest functional and structural adaption for sustained aerobic activity. Furthermore, the tuna *Euthynnus pelamis* has higher glycogen stores in white fibres (Guppy *et al.*, 1979); a reversal of the typical vertebrate pattern (Johnston, 1980b).

Bilinski (1963) found that the ability of red muscle in the same fish to oxidise fatty acids was much greater than in white muscle. A good correlation between muscle buffering capacity and activity levels is commonly observed in

different fish.

Evidence of seasonal variation also provides information. The eel *Anguilla anguilla* displays increased activities of tricarboxylic acid and certain enzymes of the hexose monophosphate shunt in its white muscle during its spawning migration (Bostrom and Johanssen, 1982).

It is not known how white fibres, which are designed for anaerobic operation, can be used during sustained swimming, as an oxygen debt is not accumulated by these fish during long periods of sustained cruising (Love, 1970).

Another possibility is that the white muscle is able to operate for long periods without tiring by a mechanism involving a rotation in the firing of the motor units to these fibres.

According to Gill *et al* (1982), there is no substantial evidence that red fibres are involved in burst swimming. However, Johnston and Tota (1974) reported that the dark muscle of Tuna (*Thunnus thynnus*) is used in high speed *sustained* swimming. These authors demonstrated that the dark muscle of these fish has a relatively high rate of contraction, and that the ATP-ase activity was as much as 50 per cent of that of its white muscle. In contrast, the ATP-ase activity of the slow-contracting dark muscle of the gurnard *Trigla lucerna* pales in comparision at only 23 per cent of that of its white muscle.

This result is typical of fish with less dynamic lifestyles. According to Mosse (1979), the white muscle of demersal species gave no histological signs of aerobic capabilities and lacked mitochondria, reflecting its capacity for exclusively transient locomotion.

Differences in metabolic and contractile profiles of different fibre types may be related to patterns of innervation.

The fact that red muscle fibres have not to date been implicated in other but a sustained role (Gill *et al.*, 1991), is reflected by the fact that they are invariably multiply innervated, and have not been shown to exhibit action potentials (Johnston, 1981, 1983).

In contrast, fast (white) fibres in teleosts may be either multiply or terminally innervated, and they are unusual in having extensive polyneural innervation (Bone, 1989; Johnston, 1981). Isolated fast fibres of this type require much higher stimulation frequencies (200-300 Hz) to elicit maximum tension than fibres with single endplates (15-20 Hz). Full activation of the fast muscles probably requires simultaneous activity of a number of polyneurally innervated fast muscles; a design which enables them to be recruited at a wider range of swimming speeds than fibres with single endplates (Johnston, 1981).

Depending on innervation pattern, a difference in electromyographic and possibly electrophysiological properties of white muscle may be observed. Teleost families can not necessarily be differentiated in terms of fast fibre innervation; most display either pattern, but Bone and Ono (1982) described both sorts of innervation in the Siluriformes, Stomiiformes and Procanthopterygii. Different patterns of innervation reflect functional differences in individual species of fish.

In the terminally innervated white muscle of elasmobranchs such as the dogfish *Scyliorhinus canicula*, there is a sharp division of labour between the red and white muscle. However, it appears that the terminally innervated white muscle of teleosts displays a spectrum of function depending on fish species. Varying degrees of aerobic and anaerobic capacities of fish fast muscle fibres have been reported according to innervation (Guppy and Hochachka, 1978; Johnston and Moon, 1980b, 1981). In higher teleosts, fast fibres are multiply innervated and may have relatively more mitochondria than the apparently entirely anaerobic fast fibres in those groups where they are terminally

innervated. Johnston (1983) observed that the mitochondrial volume and capillary density of fish white muscle innervated in the multiple pattern is up to ten times greater than terminally innervated white muscle.

This observation is supported by Raso (1991), who suggested that muscle innervation patterns of two closely related catfish with differing lifestyles could be correlated with different aerobic capabilities. Raso found that the multiply innervated white muscle of an active catfish (*Ictalurus punctatus*) had a greater aerobic capacity than the terminally innervated fast fibres of a less active counterpart.

However, whether fast fibres are multiply or terminally innervated is not necessarily an indicator of their function. The bluefish (*Romatomus saltatrix*) and the striped bass (*Morone saxatilis*) have multiply innervated white fibres which function in much the same way as terminally innervated white fibres of the dogfish and herring (Freedman, 1979).

Red fibres are invariably multiply innervated, which perhaps explains their limited versatility in locomotion. However, they have been implicated in a function of a completely different direction, one that is not related to locomotion at all.

In the course of study, certain workers noticed that the B group vitamin concentrations in superficial red muscle, which differ markedly from the white, exhibited a strong resemblance to those of the liver of the same fish. It was suggested that these structures may in fact have a parallel function (Braekkan, 1956; Mori, Hashimoto and Komata, 1956).

Braekkan (1956) argued that the high lipid content and location of the superficial red muscle made it unsuitable for serious muscular work, although the use of a cell with a great deal of contractile material for processing metabolites seemed wasteful. While Zama (1963) considered the mechanical

practicality of the red muscle to be significant, he did comment on the viability of the deep red muscle of pelagic species, in which the composition of conjugated lipid and the distribution of cholesterol was found to be similar to that of the liver.

The studies of Wittenberger and Oros (1961) concluded that a positive correlation could be observed not only between liver and red muscle lipid and cholesterol compositions, but also between the percentage of development of red muscle and the size of the liver. Their work indicated that generally, pelagic fish which have lots of dark muscle have a liver much reduced in size relative to that of a fish living on the sea bed.

While these relationships present an attractive theory, Zama (1963) pointed out that mere similarity does not necessarily constitute functional correspondence. Furthermore, Boddecke *et al.* (1959) disagreed with Braekkan's thesis, arguing that the red muscle in the pectorals of fin swimmers was identical to that seen in the superficial lateral line band, -thus locomotory muscles are also characterised by similar chemical composition to the liver. Of course, these results do not necessarily denounce either argument; but whatever the correct answer may be, there can be no doubt that red muscle fibres are indeed suitable for the metabolic function.

The red muscle-liver theory works on the pretext that red fibres remove accumulated lactic acid (in itself a high energy molecule) from the glycoltic fibres. It may then be used to reconstitute glycogen depots and to resynthesise glucose, which is subsequently released into the blood for uptake by other skeletal muscles.

Short-term use of the aerobic pathways encountered in red muscle fibres are most suitable as time enables the dissipation of lactate; otherwise it must be depleted metabolically ie converted back to pyruvic acid and then either oxidised

in the Krebs cycle or reconverted to carbohydrate stores (glucose or glycogen) (Hill and Wyse, 1989). Both metabolic pathways require oxygen which is present in substantial amounts in red muscle, and which is a consequence of the superior vascularisation. The generous flow of blood also enables rapid clearance of the lactate metabolites from the system.

The close proximity of the red to the white muscle has been cited as an obvious advantage for the transfer of metabolites, eg the exchange of catabolites for fuel (Wittenberger 1972, 1973; Wittenberger *et al.*, 1975). Studies demonstrating that lactate accumulated in the red muscle of a fish after it exercised (indicating that it had been taken up from the white) support this theory (Wittenberger *et al.*, 1975). However, according to Vyazovoy *et al.* 1982, it is more likely that lactate accumulation in red muscle is a result of increased blood flow through the red muscle during exercise. Furthermore, metabolic exchange would probably need to involve direct vascular connections between the two fibre types, which has not yet been shown to exist.

Mitochondrial involvement has also been suggested to play a part in metabolite transferral. Quaglia (1986) found that the grey mullet red muscle was composed of 80% peripheral mitochondria, possibly not involved in the contraction process (Muller, 1976), which might support the liver in oxidising lactate produced from the white fibres when working in anaerobiosis (Braekkan, 1956). Alternatively, Wittenberger *et al.* (1975) suggested that the mitochondria aid in the exchange of metabolites; possibly by means of the caveolae.

Comparisions have been drawn not only to the liver, but to the heart. There is a distinct resemblance between the lactic dehydrogenase from red muscle and that of the heart when electropherograms are compared (Matsuura, 1967).

A possible role in the seahorse

The pink oxidative fibres of the seahorse may play a role in lactate dissipation from the pink intermediate fibres. Since the dorsal myotomal white muscle is separated from the inner pink fibres by a thin band of connective tissue, it is unlikely that the white fibres are involved.

Although the oxidative pink fibres are not strictly 'red', they display many features characteristic of this muscle. (Also, the term 'red' has often been applied to oxidative muscle in general). Not only do the pink oxidative fibres stain strongly for succinic dehydrogenase, lipid and glycogen, but they are well vascularised and possess a high peripheral mitochondrial density, an important consideration in the active transfer of metabolites. Furthermore, these peripheral mitochondria do not appear to be affiliated with the contractile material. The myofibrils themselves are surrounded by other, centrally located mitochondria which may be providing the contractile supply of ATP. Also, the contractile material is not closely associated with the cell edge, but is distanced by a wide band of cytoplasm. These factors may disassociate the peripheral mitochondria from a contractile function.

The capillaries of the oxidative pink fibres were located in close proximity to the cell edge, often tucked into endothelial pockets. If these vessels are working to take up lactate from the oxidative fibres (which had been dissipated from the glycolytic muscle), then this positioning would be ideal.

Whether or not the occurring metabolic activities include the transfer of lactate from the glycolytic pink fibres to the oxidative cells and to the blood remains to be seen. The fact that the dorsal fin of the seahorse may well be producing large amounts of lactate from time to time indicates that a lactate removal mechanism would not be redundant.

Origins of the different fibre types

Pink fibres have been suggested to be a developmental stage of white fibres (Bone, 1966; Mosse and Hudson, 1977).

In the myotomes of the zebra fish *Brachydanio rerio*, the pink fibres are thought to be derived from white fibres due to their pattern of posthatching development (van Raamsdonk *et al.*, 1982). This concept has recieved support from immunohistological studies of myosin composition, which have demonstrated pink muscle to act like white muscle in reacting with 'antifast' antisera raised against white muscle myosin (Mascarello *et al.*, 1986).

Mascarello *et al.* (1986) also found that a few fibres in the region of the lateral line region of the guppy and rock goby reacted with both anti-fast and anti-slow myosin sera. Rowlerson *et al* (1975) noted this type of activity in small new fibres of some mosaic white muscle undergoing hyperplastic growth, and suggested that fibres with this immunohistochemical profile in the superficial layer of the pink muscle may be in the process of transforming into the typical pink type. However, muscle fibres with these qualities are rare in teleosts (Mascarello *et al.*, 1986).

If pink fibres are a developmental stage of white fibres, this feature would be consistent with the commonly observed pattern of transition between teleost pink and white muscle. These results should, however, be treated with caution as the staining patterns may actually be artifactual. Johnston *et al.*, (1977) showed that seasonal changes in muscle fibre type zoning and composition patterns can occur; he observed that generally, the division of muscle into zones was never as pronounced as in the summer, nor was the intensity of the stains ever as strong. Despite this issue, the fact that transition zones of various types

exist is not in question.

Most fish muscles display an abrupt transition between red and pink layers, reflecting a simple zoned pattern of fibre distribution (Bone, 1989). In others such as the grey mullet (Mascarello *et al.*, 1986), a substantial superficial intermediate zone composed of a mosaic of red and pink fibres is observed.

The most commonly observed transition pattern in teleost white-pink muscle is that of a gradual conversion, the latter becoming progressively larger approaching the white muscle and acquiring their characteristic mATPase activity (Mascarello *et al.*, 1986; Carpene and Veggetti, 1981). In the perch *Perca fluviatilis*, a pink-white transition zone was observed in which broad fibres were surrounded by narrower ones with higher activity of certain enzymes (Akster, 1985). The lateral line of the trout displays a few fibres in the pink muscle which show a more stable acid- and alkali-stable mATPase activity, which have also been described as a zone of transition (Mascarello, 1986; Johnston *et al.*, 1975b).

In transition zone fibres, the intermediate and white myosin has been shown to coexist, suggesting that these fibres are probably a separate group in their own right with a mixture of the two myosin forms (van Raamsdonk et al., 1982). This theory supports the results obtained by Mascarello *et al.* (1986).

Akster (1983) surveyed the the mosaic muscle of *Cyprinius carpio* and suggested that the gradual transition between small and large pink fibres and the occurrence of stadia intermedia between myosatellite cells and small muscle fibres implies that the small fibres are a growth stage. The fact that the number of fibres was seen to increase in number in growing fish, and that the density of nerve terminations was highest in the small fibres also suggests this possibility. Egginton and Johnston (1982b) also cite the greater fractional volume of nuclei in the small fibres as further evidence.

In salmonids, the mosaic white muscle, so called because it was first thought

to be interspersed with superficial red fibres, (on the basis of colour and lipid content) Boddecke *et al.*, 1959) was later shown to be of the same population, after the 'mosaic red' and superficial red fibre types were found to differ in terms of oxidative metabolism, myofibrillar ATP-ase activities, and electrophysiological properties (Hudson, 1973; Johnston *et al.*, 1975; Johnston and Moon, 1980). These workers came to the conclusion that the small mosaic fibres actually represented the earlier growth stages of the same white fibre population.

Growth, involving an increase both in size and number of cells has been observed in many fish. It has been demonstrated in cod (Greer Walker, 1970) eels (Willemse and van den Berg, 1978) mullets (Carpene and Veggetti, 1981) and salmonids (Davison and Goldspink, 1977; Weatherley *et al.*, 1979, 1980; Weatherley and Gill, 1981). Carpene and Veggetti (1981) proposed that the white fibres increased in number by the addition of new, small fibres to the existing population, which could be identified by differences in staining for various histochemical techniques. However, Davison (1982) did not observe this mosaic pattern even though the study animals were collected during periods of rapid growth.

Nag and Nursall (1972) and Kryvi *et al.* (1977) suggest that in teleosts new muscle fibres arise from myosatellite cells. The presence of myosatellite cells and of transition stages between myosatellite cells and small muscle fibres in *Cyprinius carpio* (Akster, 1983) strongly supports the theory of the small fibres being a growth stage.

Although evidence is accumulating to show that new fibres are derived from the differentiation of satellite cells (Weatherley *et al.*, 1979, 1980; Carpene and Veggetti, 1981), a further hypothesis has suggested that new fibres originate from cell division (van Raamsdonk *et al.*, 1982). In mammalian muscles, increases in

cell numbers are due to the splitting of existing fibres (Rowe and Goldspink, 1968; Gonyea, 1980). The number of muscle fibres is determined shortly after birth (Goldspink, 1982). Any increase is muscle cell numbers is produced by grossly overloading the muscle.

If increases in cell numbers is due to transformation of satellite cells, then growth of fibres in fish possessing this feature must be rapid. In the present study, myosatellite cells were not seen at the light microscope level, indicating that growth occurs by hypertrophy of fibre diameter. This suggestion is supported by the observed increase in cell diameters in all fibre types. Also, the lack of a transition zone between the white fibres and the pink fibres in the myotome of the dorsal fin provides further evidence that the pink fibres are not derived from the white.

The origin of the small diameter fibres has not been approached in great detail, as these fibres have really been investigated very little (Davison, 1993 pers. comm.). However, small diameter fibres have been suggested to be a growth stage of red fibres.

Proportions of fibre types

The proportions of each fibre type in the myotome are variable among teleosts. Greer-Walker and Pull (1975) showed that percentages of red muscle in fish myotomes varied from 0% (in five fish families) to 26% in the Scombridae. The proportion of oxidative muscle (especially red) tends to be greater in fishes such as salmon in which sustained cruising is an important feature, while fish that rely on other modes of locomotion show a reduction in myotomal oxidative musculature (Greer-Walker and Pull, 1975; Mosse and Hudson, 1977).

In those teleosts which use their fins for locomotion, the oxidative muscle is usually well developed, while the myotomal proportions may be reduced (Davison and Macdonald, 1985; Kilarski *et al.*, 1982; Davison and MacDonald, 1985). In some specialised teleosts such as the leatherjacket *Pariker scaber* which swims using its well developed dorsal and anal fins, the red muscle has been lost altogether (Davison, 1983a; Davison and MacDonald, 1985; te Kronnie *et al.*, 1983). The myotome of this fish contains essentially only one muscle type (white), used for high speed swimming, although the fin muscles contain a typical array of peripheral red, intermediate pink and deep white fibres (Davison, 1987). Pink muscle in the myotome is thought to be vestigial.

Although oxidative muscle has not been implicated in burst swimming, the presence of oxidative muscle in the myotome of a fish which does not use its tail for slow speed swimming is not unique (Kryvi and Totland, 1978; Starling, 1989). Walesby and Johnston (1980) have described an Antarctic fish which swims in a labriform manner while posssessing a substantial amont of red muscle. It is presumed that this fish must still posssess the ability to swim slowly using its

lateral muscles.

In other fish like the labriform spotty *Pseudolabrus celidotus*, levels of myotomal oxidative musculature are reduced but not eliminated, since the myotome is still recruited for burst swimming (Tulloch, 1990).

The proportion of oxidative musculature in the dorsal fin of the seahorse was larger than many of the myotomal values presented by Mosse and Hudsons' extensive survey (1977). (Few studies have concentrated on fin muscle proportions). The large proportions of dorsal oxidative musculature are parallelled by the lack of it in the myotome, indicating that a substantial shift in locomotory emphasis from the myotome to the dorsal fin has occurred. The proportion of pink oxidative fibres in the locomotory musculature is probably a consequence of three factors: the high speeds of undulation observed in the dorsal fin requiring large numbers of slightly glycolytic fibres; the fact that such fibres are bigger than red fibres (which usually constitute measurements of oxidative muscle proportions); and the lack of propulsive contribution from the myotome.

The proportions of oxidative musculature were seen to change with increasing fish length. Although Hudson (1973) found a negative correlation between fish size and proportion of oxidative muscle; in this study, a positive correlation was seen in the dorsal oxidative fibres. However, a negative correlation was observed between fish size and myotomal tonic musculature.

The actual amount of oxidative muscle present has been shown to increase when fish are acclimated to lower temperatures (Malessa, 1969: *Anguilla anguilla*;) Wodtke, 1974), and in the carp *Carassius auratus* (Johnston, 1976). Differences in amounts of dark muscle have also been reported from different sites in individual species. Greer Walker (1970) observed a slight increase in

fibre diameters of *Gadus morhua* taken from regions increasingly near the tail, and Frontier-Abou (1969: *Caranx ignobilus, C. sexfasciatus* and *C. stellatus*) found that while the proximate composition of white muscle is essentially the same from one end of the animal to the other, the dark muscle contains more lipid in the anterior part of the fish and conversely more water and protein in the posterior part. During periods in which the fish is able to replenish its fatty stores, it is the anterior dark muscle that accumulates most of the lipids.

The white muscle fibres in the dorsal fin of the seahorse were observed to decrease in proportion with increasing fish length, as the proportion of oxidative musculature increased. This suggests that it is the oxidative musculature which is primarily involved in locomotion, while the white fibres are recruited very rarely. The degeneration of the dorsal glycolytic 'fast' musculature in favour of an increase in oxidative locomotory musculature supports the earlier visual observations which indicated that the seahorse is incapable of rapid bursts of speed. The reduced dorsal fast muscle proportions may be a consequence of the possibility that these fibres are not frequently recruited in locomotion.

In spite of the fact that oxidative muscle proportions exceeded those of the glycolytic fibres in the dorsal fin; white muscle fibres made up the bulk of the seahorse myotome. In this part of the seahorse, white fibres are also suggested to be recruited rarely; possibly for lashing movements elicited when the animal is seized by an aggressor. These fibres may also be recruited to grip the substrate hold during such adverse circumstances, if the tonic fibres cannot maintain it. Another possible role of the white fibres could be to mediate the rudder-like movements occasionally observed.

This pattern of occasional recruitment of large masses of glycolytic muscle is commonly observed in teleost fish. In terrestrial animals as we have seen,

there is a considerable penalty for carrying round a large mass of muscle that is only for intermittent use; however the buoyant nature of the aquatic medium determines that fish are not constrained by this limitation. This feature provides scope for the development of large proportions of muscle. Even so, the slow-contracting red fibres usually constitute a small proportion of the total musculature, simply because they rely on a good supply of oxygen to power their aerobic energy pathways, which is limited by the surface area of the gills.

White muscle fibres are not constrained in this way as they function anaerobically (Bone *et al.*, 1978; Johnston *et al.*, 1977; Kilarski *et al.*, 1982). Typically, they constitute up to 90% of fish muscle, depending on species, giving it its characteristic colour and culinary importance (Mosse and Hudson, 1977). These proportions are in fact necessary for the efficient operation of the glycoltic musculature. Since the force of contraction is directly proportional to the numbers of fibres recruited, large numbers are required for effecting rapid bursts of speed (Lindsey, 1978). In addition, as fish swimming speed escalates, the drag of the water on the surface of the skin increases to the cube of velocity; thus as the fish increases its velocity, further fibre recruitment is necessary (Lindsey, 1978).

Tonic fibres are not thought to play a role in locomotion, thus they are usually present in small numbers (Davison and MacDonald, 1985; Johnston, 1981; te Kronnie *et al.*, 1983; Morgan and Proske, 1984). In the myotome of the seahorse, however, the proportion of tonic fibres ranges from 23-30 per cent; a much higher proportion than most fish of a more typical design, in which an average of 0.5% is observed (Kilarski and Koslowska, 1986).

The uniquely high tonic fibre proportions are undoubtedly a product of the fact that the tail of the seahorses and pipefishes stands alone in terms of structure and function. In a 'typical' fish (i.e. without a prehensile tail), tonic

fibres play a relatively minor role in the myotome; thus they are present in significantly smaller proportions than the locomotory muscle proper. However the tail of the seahorse is certainly not used in propulsion; therefore tonic fibres do not compromise twitch muscle proportions by taking up space, as there is no locomotory use for it. In comparision, there is plenty of use for fibres which enable it to maintain its hold on the substrate without the expenditure of large amounts of energy. The distal part of the tail displayed a greater proportion of tonic musculature than the mid part of the tail, which is logical as the distal part of the tail is the most flexible, being the point of contact with the mooring. This observation provides further evidence for the argument that the tonic fibres of teleost fish are analogous to those of mammals, which have a postural function.

Percentages of cell components

Described differences in the relative volume of the cell components are not consistent (Akster *et al.*, 1985).

Quaglia (1980) found that the white and red fibres of the grey mullet *Mugil cephalus* did not vary in terms of sarcoplasmic reticulum and T-system. However, red fibres were found to be richer in mitochondria than white fibres, a result which supports the view that the red and white fibre types play different roles in swimming locomotion. According to Patterson and Goldspink (1972), most teleost muscles show proportional variations not only in mitochondrial percentages, but in myofibril and sarcoplasmic reticulum densities, depending on species and fibre type. According to Johnston (1980b), fractional volume occupied by myofibrils in teleost fishes varies from 80-90 per cent in white muscles; and from 40-69 per cent in red.

In the seahorse, the pink oxidative fibres contained nearly half the relative volume of myofibrils of the pink intermediate fibres. The difference between the myofibril proportions of the two pink fibre populations may be explained by the fact that a larger proportion of the total cell space in the oxidative fibres is taken up by mitochondria; whereas the glycolytic fibres rely instead on an energy pathway in which larger proportions of muscle are preferentially recruited in order to maximise power output.

According to Akster (1985), the difference between the myofibril proportions of both fibre types can also be interpreted to mean that the oxidative fibres can achieve approximately one-third of the maximal tension of the intermediate population.

Percentages of mitochondria were found to be higher in the dorsal pink oxidative muscle fibres than in any other fibre type. Differences in the relative volumes of mitochondria may in all cases be correlated with the observed histochemical differences in oxidative enzyme activity, also reported by Akster (1983).

In the carp *Cyprinius carpio* (Akster, 1985), both red muscle and oxidative pink muscle mitochondrial densities were less than oxidative muscle mitochondrial densities of the seahorse. The same trend may also be observed in comparisons to other fish mitochondrial densities (See Table 4).

The fact that pink oxidative mitochondrial densities were unusually high in *Hippocampus*, even compared to values for red fibres, was not expected, as pink fibres typically have a mitochondrial content intermediate between red and white fibres (Johnston and Goldspink, 1980). However, the larger relative proportions of mitochondria observed in the oxidative pink dorsal fibres undoubtedly serve to increase the oxidative capacity of these fibres.

Walesby and Johnston (1980) and Davison and MacDonald (1986) suggested that the oxidative capacity of the red muscle in the myotome of fin swimmers is reduced compared to that of the fin muscles. This pattern is demonstrated to the extreme in the seahorse, in which the unusually high proportions of mitochondria in the locomotory pink muscle fibres was reflected by the complete lack of it in the myotome.

The dorsal intermediate pink fibres had only a slightly superior percentage of mitochondria than the white fibres of the seahorse dorsal fin; a fact which reflects the corresponding energy pathways employed by these fibres. The slight difference may be attributable to the fact that white fibres are not involved in locomotion.

Fish red muscle mitochondrial density often exceeds that found in the most

active mammalian muscles. The fractional volume found in red muscles of various cold-water species is comparable to the volume in the ventricular muscle of the mouse (34%) and finch (37%) (Bossen, Sommer and Waugh, 1978). In contrast, only (approximately) 0.5-0.8% of white fibre volume is occupied by mitochondria, depending on species and position in the myotome. The numerous mitochondria seen in the pink oxidative muscle fibres of the seahorse reflects that oxidative phosphorylation and fatty acid oxidation are carried on actively in the citric acid cycle in these structures, and that ATP production is high in this muscle.

A large relative mitochondrial volume and glycogen content tends to correspond with good endurance capabilities (Akster, 1985). This is unlikely in the seahorse, however, as the fish is incapable of swimming for sustained periods of time.

The ratio of mitochondrial numbers versus cell volume has been seen to vary between species, even within species with varying locations and effects, for example season (Johnston and Bernard, 1982a,b; Johnston, 1982), and difference in water temperature (Johnston, 1982). Hypoxia may also influence the volume of mitochondria within a species, as it modifies volume density of mitochondria and capillarisation in swimming muscles of fish (Johnston and Bernard, 1982a,b, 1984). After a period of acclimation to hypoxia there is an increase in the efficiency of oxygen extraction; in some species the response is reduced spontaneous locomotory activity to decrease the demand for oxygen (Lomholt and Johansen, 1979; Johnston *et al.*, 1983). In others such as the crucian carp, an increase in slow muscle mitochondrial volume density is observed (Johnston and Bernard, 1984). Differences in mitochondrial volume density within species are adaptions to habitat variations which enable the maintenance of sufficient oxygen supply to the tissues, and reflect each individual species behavioural,

cardiovascular and respiratory adjustments to low partial pressures of oxygen (Johnston and Bernard, 1984).

These factors may also affect myofibril density and structure. Sänger *et al.* (1990) recorded that the fine structure of muscle fibres in roach (*Rutilus rutilus*) and chub (*Leuciscus cephalus*) differed within-species and between localities.

Patterns of innervation have been suggested to influence mitochondrial densities, although this relationship may be indirect. Johnston (1983) observed that the mitochondrial volume and capillary density of fish white muscle innervated in the multiple pattern is up to ten times greater than terminally innervated white muscle.

Shindo *et al.* (1986) reported that in all species studied, with the exception of *Tilapia*, far less mitochondria were seen in the white fibres compared to the red, although he did not quote percentages. This pattern is certainly observed between the pink oxidative and white glycolytic fibres of the seahorse. No significant differences were seen between any of the myotomal myofibril percentages, for either white or tonic fibres. The same trend was observed for mitochondrial and correspondingly cytoplasmic densities in these regions of the myotome. These results show that the tonic and fast twitch muscle fibres of the seahorse are similar in this respect. In terms of contraction speed and functional capabilities, they are highly unlikely to be comparable.

These results are somewhat exceptional, as myofibril volumes in twitch muscles are generally lower than those of tonic fibres in *Noemacheilus barbatulus* (Kilarski and Koslowska, 1986). Kilarski and Koslowska (1986) also note that the volume density of mitochondria in the tonic fibres of *Noemacheilus barbatulus* was nearly 4% ; whereas the seahorse tonic fibre mitochondrial density was significantly less, (2.1 ± 0.8: mid tail; 2.0 ± 1.2: distal tail). These differentiated patterns are undoubtedly a result of the differentiated role played by the tonic

fibres in the seahorse in comparison to other fish.

The lack of mitochondria observed in the white fibres of the seahorse is a consequence of the anaerobic energy pathway employed, and is reflected by the high stability of their mATPases and low levels of oxidative enzymes. Since the power produced by a given amount of muscle depends on the actin-myosin interaction, any modifications which will allow more myofilaments in a given volume will increase the power output. Correspondingly, white muscle fibres have very few mitochondria interrupting the myofilament array, and few muscle capillaries occupying space that could be filled by muscle fibres.

Nag (1972) showed that the white muscle of *Salmo gairdneri* contains nearly three times as much sarcoplasmic reticulum in relation to myofibrils as the dark muscle. In the seahorse *Hippocampus hudsonius*, Bergman (1964) reported a 'uniquely high' ratio of sarcoplasm to myofibrils of 25:75; a similar pattern to *Hippocampus abdominalis*.

A sarcoplasmic reticulum with a very large surface area can be related to increased calcium uptake during the contraction of the myofilaments. The rate of contraction is limited by specific calcium binding and the transportation activity of the membranes of the sarcoplasmic reticulum; also the surface area of the reticulum relative to the sarcoplasmic volume (Nag, 1972).

Fibre diameter implications

The various fibre types in the dorsal fin and myotome of the seahorse reflect the different functional roles that they play. However, variations in cell diameters depend on age, sex, fitness and condition (Chayen *et al.*, (1987). According to this author, fibre sizes remain much the same from season to season; however, atrophy of cells due to malnutrition probably occurs in some fish during the winter months due to insufficient nutrients present to maintain the integrity of the cell.

The variation observed in the dimensions of pink oxidative fibres is parallelled in their metabolism. Diversity of size depends on the location of the fibres, with larger fibres adjacent to white muscle showing weaker oxidative enzyme activity; while those adjacent to red fibres stained positively for this test. Therefore, the smaller oxidative pink fibres probably have a greater aerobic capacity than the larger ones located further from the fulcrum. The small size of the oxidative pink fibres is an important factor in aerobic metabolism as oxygen diffusion distances are shorter, enabling optimal operation.

A possible explanation for differences in oxidative pink fibre diameter size comes from experiments on fish exercise (Greer-Walker and Pull, 1973; Davison, and Goldspink, 1977). The influence of exercise on fish muscle fibre diameter is not yet clear. Experimental studies have shown endurance training of trout produces increases in the number and diameter of the red muscle fibres (Davison and Goldspink, 1977). Smaller fibres in unexercised fish may be a result of atrophy due to infrequent use (Greer-Walker and Pull, 1973); or an attempt to increase the oxidative capacity of the cell by reducing the oxygen diffusion distance (Smialowska and Kilarski 1981).

In mammals, the opposite trend is commonly observed. Increases in fibre size are noted during periods of inactivity, while decreases are observed after prolonged exercising (Houston *et al.*, 1979). A similar parallel is seen in some teleosts such as the spotty *Pseudolabrus celidotus*, in which dorsal oxidative fibres were smaller than their myotomal counterparts (Starling, 1989). This trend is proposed to occur because dorsal oxidative fibres are constantly recruited; thus they are required to have a greater aerobic capacity. The maintenance of short oxygen diffusion distances is essential in order to enable maximum exploitation of aerobic pathways. When use of oxidative locomotory fibres is sporadic, fibres tend to be bigger. The range in locomotory fibre size seen in the dorsal fin musculature of the seahorse probably reflects an adaptive measure, to have a choice of fibres of varying size and hence oxidative capacity available for recruitment according to requirement. Variation in myotomal white muscle fibres is probably a consequence of the fact that they are not limited in terms of oxygen diffusion, a feature which allows scope for the development of different fibre sizes.

The larger size (and hence greater oxygen diffusion distances) of the pink intermediate fibres reflects their anaerobic capacity. Although they also had quite a variable range of fibre diameters, they were probably not as diverse in terms of metabolism, as they did not show differential staining for succinic dehydrogenase, glycogen or lipid. The larger size of these fibres is probably less likely to be a consequence of sporadic use than of functional requirement, although it is difficult to assess what proportion of the locomotory contribution these fibres actually represent.

Fast fibres in the dorsal fin of a 29 cm seahorse showed an interesting trend, in that a maximum fibre size was being reached at this length. The increase in fibre diameter appears to 'tail off' at a certain level, at the very large

fibre size of approximately 250 μm. This is not actually surprising, since although fast fibres are not generally cited as being limited by oxygen diffusion distances, there surely must come a point where they can no longer get any bigger. It appears that this point may be at around fibre diameters of this proportion.

The fact that dorsal fast fibres are, on average, from a third to half as large again as the myotomal fast fibres may suggest that these fibres are being recruited for strenuous bursts of locomotory energy, such as a 'last resort' when the aerobic and glycolytic pink fibres have been exhausted. Alternatively, the fact that the total surface area of the seahorse myotome is much reduced in comparision to the dorsal fin surface area may explain the size differentiation.

The tonic fibres of the seahorse varied considerably, both in the dorsal fin and more significantly, the myotome. The large range of teleost tonic fibres is rarely reported in fish. Kilarski and Koslowska (1985) reported that the small diameter fibres of *Noemacheilus barbatulus* are predominantly small in diameter, ranging form 8-24 μm. In contrast, the SDF of the seahorse dorsal fin ranged from 25 μm ± 6 (length=19 cms) to 38 μm ± 9 (l=29 cms). Even more differentiated were the myotomal tonic fibres.

Kilarski *et al.*, 1985) found that the tonic fibres of *Noemacheilis barbatulus* had a mean diameter of 17.08 ± 0.77. These values are far smaller than those for the seahorse myotomal tonic musculature; however these discrepancies can be explained by the fact that tonic fibres play a significantly different role in the myotome of the seahorse than in the aforementioned fish. Apart from the Syngnathidae, no other fish families possess prehensile tails; thus it is logical that the pattern of tonic fibre diameters seen in the seahorse is unique. The variation in tonic fibre sizes may correspond to varying degrees of grip exerted by the tail; alternatively, it may exist simply because the potential scope for varying

dimensional size is not limited by oxygen diffusion distances or functional space in the myotome.

Many workers have considered the range of fibre size in teleost white muscle to represent different stages in growth rather than distinct fibre types (Johnston *et al.* 1975; Dahl and Paulson, 1978). However this would seem to ignore the physiological differences in metabolic, contractile and elemental profiles. Further evidence to the contrary is the positive correlation in some fish (including the seahorse) of increasing length with fibre diameter, indicating that the fish are growing by fibre hypertrophy rather than by producing new fibres from myosatellite cells. However, Greer-Walker and Pull (1970) have found that fibre number does increase throughout life in a number of fish, particularly fast growing species. Cell hypertrophy is more commonly seen in slow-growing species (Weatherley and Gill, 1981, 1984). This indicates that the seahorse is a slow-growing species. As no information is available concerning growth and maturity factors in these fish, it is not possible to confirm or deny this assumption.

Some workers have proposed that interspecific differences can be related to physical form. Stickland (1975) suggested that more streamlined fish have smaller diameter fibres. This may be an indirect correlation, as streamlined fish are frequently more active and so have higher levels of metabolism which require shorter oxygen diffusion distances for sustained activity. The work of Smialowska and Kilarski (1981) demonstrated an inverse relationship between metabolic activity and the diameters of both red and white fibres; however Kilarski *et al.* (1982) could not demonstrate a relationship between the diameter of muscle fibres and streamlinedness, and suggested that the effect of metabolism was the overriding influence.

Summary

The seahorse is a unique animal which is adapted to a highly specialised mode of life; in terms of morphology, physiology and locomotion. The evolutionary loss of the typical piscean streamlined shape, and the forfeiture of speed in favour of manoeuverability, have doubtless evolved in parallel with the observed modifications in myotomal structure and function.

Profiles of individual muscle fibre distributions and characteristics are reflected by these features, in that they are also highly unusual in comparision to typical fish.

One of the most significant features observed in the seahorse musculature was the complete lack of mATPase labile red fibres from both the locomotory and myotomal musculature. This pattern of fibre distribution is not unique (Tulloch, 1990); however it does represent a diversion from the original piscean archetype (Bone, 1989). The widely accepted concept of functional specialisation of the myotomal musculature into two specialised fibre types has been argued to be a homologous feature of all fish groups (Bone, 1989). The implication of this theory is that red fibres were at some stage present in the musculature of these strange little fish. The actual mechanism behind the loss of red muscle from the seahorse musculature is unknown; however the functional replacement of the slow fibres by a population of tonic muscle in the myotome, and by pink fibres in the dorsal fin, clearly indicates an evolutionary response to the requirements imposed by a highly specialised mode of life.

Assuming that each fibre type has a different role, rather than constituting simply a growth stage of other fibre types (Mosse and Hudson, 1977); the

operation of the dorsal fin is effected by four functionally disparate fibre types; the pink oxidative fibres, the pink intermediate fibres, the white fibres, and the tonic fibres. The operation of the myotome, in contrast, is effected by just two; white and tonic fibres.

In the dorsal fin, the presence of a large proportion of mATPase stable pink muscle was noted. According to Walesby and Johnston (1979) and Johnston (1985), pink fibres are not suitable for maintaining sustained swimming, due to their fast contraction speeds and high mATPase activity. However, in the seahorse, locomotion is certainly not sustainable, and furthermore, the operation of the dorsal fin probably requires the recruitment of fibres with a higher speed of contraction than that which is characteristic of red muscle. Additionally, the fact that short distance movements (which are typical of seahorse behaviour), are thought to require relatively fast contracting fibres to overcome inertia and gravity also discredits the red fibres as a suitable contractile fibre choice.

On the basis of these deductions, it may logically be suggested that the archetypal slow red fibres have been eliminated from the dorsal fin musculature because they were unable to meet the energy demands of the contractile apparatus without generating an oxygen debt.

Pink muscle fibres are functionally recruited at locomotory speeds intermediate to those powered by contractions of red (slow) and white (fast) fibres (Johnston *et al.*, 1977). In view of the locomotory profile of the seahorse, these fibres are probably better suited to operate the dorsal fin. What does complicate the issue, however, is the extent of the mitochondrial density and vascularisation in the oxidative pink fibres, which implies that the aerobic capacity of these fibres is comparable to the red fibres. The fact that mitochondrial numbers in these fibres are unusually high suggests that the appeal of pink fibres lies not in their (typically moderate) aerobic capacity, since

this factor has been increased; but in the contractile characteristics of this muscle.

The fact that the generated force of contraction is proportional to the area of contractile units recruited also explains why the proportion of oxidative musculature in the seahorse is comparitively large. The seahorse probably employs the oxidative pink fibres in moving from perch to perch in its typical unhurried style, using either lipid or glycogen as an aerobic substrate depending on metabolic requirements at the time.

The role of the mATPase labile pink fibres is harder to define. Pink muscle has typically been classed as oxidative (Johnston, 1980; Goldspink, 1983). However, while this observation is certainly supported in the case of the mATPase stable fibre population, the unsubstantial mitochondrial content of the intermediate pink fibres implies that they are glycolytic. Since these fibres were present in much smaller numbers than the mATPase stable pink fibres, it may be unlikely that they could produce a significant locomotory contribution, since glycolytic fibres are generally recruited in large proportions to effect the propulsion. Alternatively, the fact that the dorsal fin as a propulsive device is quite small in comparison to other fish locomotory structures could indicate that a population of fibres of these proportions would be entirely adequate. The intermediate pink fibres of the seahorse are probably employed at speeds just under the threshold for white fibres, having the effect of sparing the white fibres and avoiding the accumulation of lactate associated with an oxygen debt (Johnston *et al.*, 1977).

It is not really possible to hypothesise whether the contraction speeds of the functionally disparate pink fibre populations are differentiated, although the fact that mATPase lability is typically indicative of slow-contracting muscle fibres could implicate that the mATPase stable fibres contract more rapidly (Barany,

1967).

Tulloch (1990) observed the same differentiated pattern of staining in two populations of pink fibres of *Obliquichthys maryannae*; and noted that the intermediate pink fibres in this fish were more similar in terms of ultrastructure to the white fibres. However, the results obtained from ultrastructural analyses of the seahorse muscle do not reflect this pattern, in which both populations of pink fibres were identical to each other, but dramatically different in terms of myofibrillar ultrastructure to the white fibres.

The pink fibres undoubtedly possess a greater degree of resistance to fatigue than white fibres, due to their higher mitochondrial content (Johnston, 1981). Their recruitment probably enables an increase in power output while conserving energy of the white fibres.

White fibres in the dorsal fin were observed to be present in two identically staining populations. The fact that the small white fibres were present in such low numbers and at the position generating the least 'torque' indicates that they are most unlikely to play a significant role in locomotion. In contrast, the large dorsal fibres are well suited to locomotion of a transient type. The recruitment of the large white fibres in the dorsal fin may enable an increase in frequency and power of fin beats; alternatively, these fibres may provide a source of power as a last resort, when the energy reserves of the pink fibres have been depleted.

White fibres have also been proposed to be important in fish rising from a benthic position, and in overcoming inertial forces and drag during short distance movements requiring a large initial energy expenditure (Lindsey, 1978). According to Webb (1975); the effect of drag at slow speed is negligable; therefore a small population of white fibres could theoretically be sufficient to change the fishes state of inertia. Davison and MacDonald (1986) proposed a similar role for the white fibres in the flexor muscles of the labriform fish,

Trematomus bernacii, a benthic Antarctic fish. However, the suggestion that the small population of white fibres may produce the necessary power output for such operations is questionable. Alternatively, the fact that they are closely associated with the small diameter tonic fibres adjacent to the fin rays implicates them in a supportive role. Furthermore, the slow speeds at which the seahorse moves indicates that drag probably never becomes a consideration, despite the loss of streamlining.

During locomotion, fibres of different contractile speeds are probably selectively recruited to enable optimum exploitation of the available musculature. Elevation of the power output or amplitude of the dorsal fin stroke requires the recruitment of a proportionally greater number of fibres regardless of contraction speed as power output is proportional to the number of contractile units recruited (Bone, 1975).

Although the proportions of white fibres in the myotome of the seahorse are reduced, they still constitute the bulk of the myotome, and in this respect they conform to the average. Teleost white fibres are clearly adapted for fast speed swimming (Hudson, 1973; Johnston *et al.*, 1977). They also function to 'pack out' the myotome, providing a maximum surface area with which to push against the water -an important functional consideration in fish which employ carangiform locomotion (Lindsey, 1978). However, in the seahorse; neither of these considerations are relevant. The fact that the myotome is not employed in locomotion denotes that the constituent white fibres certainly play no role in propulsion.

The adaption of the seahorse myotome to a prehensile, non-locomotory form has reduced the necessity of such large proportions of white fibres. This is reflected in the substantially reduced myotomal area of the tail; a feature which has also been facilitated by the prehensile development of the tail. The tail is

narrowest and most flexible at its tip, which is the region of grip.

Although the seahorse myotome is used as a balancing device with which to change the centre of gravity as it hovers, it is not observed to be employed very often. The seahorse is rarely observed to hover or swim about, except when moving from perch to perch, preferring to attach itself to the seaweed or substrate on which it is browsing; thus the tail rarely functions as other than an anchoring device.

White fibres are unlikely to play a role in the postural functions of bending the tail, or locking it in a grip around a suitable substrate, without the expenditure of uneconomically high expenditure of energy. During normal periods of attachment, the myotome was not observed to fatigue in any way. This fact in itself rules out the possibility of the white fibres playing a role in this function.

In contrast, the metabolic and structural profiles of the tonic fibres are ideal for this purpose. At the ultrastructural level, small diameter fibres have been shown to have a poorly developed sarcoplasmic reticulum and relatively few mitochondria (Kilarski and Koslowska, (1985), which fails to implicate them in either a sustained or a transient swimming role; furthermore, their very small size and characteristically low proportional densities in which they are found denotes that it is unlikely that they could play a locomotory role of any description at all. The tonic fibres in the dorsal fin of the seahorse support these findings; however, those found in the myotome present a rather different picture. Not only were they characterised by a large, variable dimensional range, but they were observed to have a well-developed sarcoplasmic reticulum, a feature which is often associated with faster-contracting fibres. The differences in staining characteristics, ultrastructure, and fibre sizes of the tonic fibres in the dorsal fin and the myotome all reflect the fact that differentiated roles require different

metabolic profiles.

The characteristics of the tonic fibres in the myotome of the seahorse were greatly differentiated in comparison to tonic fibres in other fishes. This disparity, along with other unusual features of *Hippocampus*, may be related to the unique mode of life of this animal.

The results of this study appear to have confirmed that tonic fibres in the myotome of this particular teleost are indeed analogous to the postural fibres of mammals, as proposed by Johnston (1985); Davison (1983a,b); and Davison and MacDonald (1985). However, a definite gap exists in the literature regarding the electrophysiological and physiological properties of the tonic fibres; once these have been confirmed, then it may be possible to confirm or deny the proposed roles of the tonic fibres.

The relationship between the body form of the seahorse and its locomotion is complex. Physical form largely determines the ability of the fish to escape predators and to forage (Webb, 1984a). The structurally complex habitat occupied by the seahorse is most efficiently exploited by specialists in median or paired fin propulsion; a locomotory form optimally suited to niches where the prey does not attempt to escape, but occupies a habitat in which the exploitation of prey requires accurate and complex manoeuvering by a predator. In order to exploit the characteristics of its structurally complex habitat, the seahorse has evolved a manoeuvering competence far superior to most teleost fish. This study has shown how the seahorse is adapted to its specialised mode of life; and that the unusual muscle fibre profiles which have been defined represent an optimal set of characteristics which enable the maximal exploitation of this interesting creature's mode of life.

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